Difco™ & BBL™ Manual

Manual of Microbiological Culture Media

Second Edition

BD

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Manual of Microbiological Culture Media

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Preface

We are pleased to present the second edition of the *Difco & BBL Manual*. The first edition, published in 2003, replaced the *Difco Manual*, 11th ed. (1998) and the *Manual of BBL Products and Laboratory Procedures*, 6th ed. (1988), manuals which were first published in 1927 and 1948, respectively. The *Difco & BBL Manual* is devoted exclusively to culture media and associated reagents offered by BD as **Difco**™ and **BBL**™ brands of dehydrated culture media and **BBL**™ brand prepared plated, tubed and bottled media. In this manual, over 170 years of combined media experience is brought together in an educational and reference text.

For the second edition of the *Difco & BBL Manual*, we have added new products, removed discontinued products and incorporated general updates throughout. Of special note, the descriptions for media affected by the harmonization of the pharmacopeias (United States, European and Japanese) have been updated accordingly.

The reader is advised that these products are for use by or under the supervision of microbiologists and other professionals qualified by training and experience to handle pathogenic microorganisms and samples and specimens containing or suspected to contain them. Also, it is expected that the user will be thoroughly familiar with the intended uses of the formulations presented and will follow test procedures outlined in the applicable official compendia and standard texts or the procedure manual of the using laboratory.

In addition to providing this manual as an educational resource, BD Diagnostics offers an array of educational materials and services:

- BD Bionutrients™ Technical Manual – a manual dedicated to products used in cell culture and microbial fermentation production.
- BD *LabO*™ – a newsletter providing the latest microbiology news and ideas to the clinical laboratory.
- Technical and Product Support – a dedicated group of specialists available to answer questions about any of our products or procedures.
- Our web site, www.bd.com/ds

Grateful acknowledgement is made of the encouragement and support received from microbiologists throughout the world. Our appreciation is extended, also, to those who have contributed their talents and time to the creation of this manual and its predecessors. It is our desire to continue to extend our services to the advancement of microbiology and related sciences.

Becton, Dickinson and Company

About This Manual

The *Difco™ & BBL™ Manual* presents in one volume descriptions of the media currently offered by BD as **Difco™** and **BBL™** brands of dehydrated culture media and **BBL™** brand prepared plated, tubed and bottled media. Since products and labeling are being reviewed continuously, the information provided in this manual is superceded by current product listings and labeling, when differing from descriptions in this manual (e.g., product labels, package inserts, certificates of analysis, etc.). For product availability, consult our product catalog (online) or contact your local distributor or BD representative.

This manual is organized into four major sections:

1. Monographs pertaining to the development, quality control and utilization of microbial culture media.
2. General technical information on dehydrated and prepared culture media.
3. Product descriptions for culture media and ingredients.
4. Reference section containing tables summarizing industrial and clinical applications and descriptions of related media and media component analyses.

Product descriptions for dehydrated culture media contain the following sections:

Intended Use
Summary and Explanation
Principles of the Procedure
Formula(e)
Precautions (as applicable)
Directions for Preparation from Dehydrated Product
User Quality Control
Procedure
Expected Results
Limitations of the Procedure (as applicable)
References
Availability

As highlighted in the Preface, the descriptions for media referenced in Chapters <61> and <62> of the recently harmonized *United States Pharmacopeia* have been updated. Specifically, the section on "User Quality Control" contains the information required to verify that these media were tested according to the *United States Pharmacopeia, European Pharmacopoeia* and *Japanese Pharmacopoeia* – **for both dehydrated culture media and prepared culture media products**. In other words, the media listed under "Availability" and identified with a staff mark (†), have been tested and meet *USP, EP* and *JP* performance specifications where applicable. Also note that for these media, the "Formula" provided is the same for both dehydrated and prepared media products.

For media offered only as prepared plated, tubed or bottled media, the descriptions are abbreviated; complete descriptions, including the sections for "Formula" and "User Quality Control," are provided in accompanying package inserts or the ***BBL™*** *Quality Control and Product Information Manual for Plated and Tubed Media*. This manual is available online at http://www.bd.com/ds/technicalCenter/inserts/qcpiManual.asp or upon request from BD Technical Services (hard copy).

American Type Culture Collection strains (ATCC™), or commercially available derivatives of these strains, are specified for performing quality control procedures on laboratory-prepared (and commercially-prepared) media. When listed in the "User Quality Control" section, organism names generally conform to ATCC labeling. In text, abbreviated names may be used; e.g., serotypes of *Salmonella enterica*.

This manual includes international catalog numbers and standard methods icons. Under "Availability," catalog numbers are provided for prepared plated media manufactured in Europe, Japan and Mexico that are comparable to the formulations manufactured in the United States. In addition, icons denoting media listed in "official" and "standard methods" publications are provided. These icons represent:

AOAC Official Methods of Analysis of AOAC International[1]
BAM Bacteriological Analytical Manual (FDA)[2]
CCAM The Compendium of Analytical Methods (Canada)[3]
COMPF Compendium of Methods for the Microbiological Examination of Foods (APHA)[4]
EP European Pharmacopoeia[5]
EPA Manual for the Certification of Laboratories Analyzing Drinking Water (EPA)[6]
ISO International Standards Organization[7]
JP Japanese Pharmacopoeia[8]
SMD Standard Methods for the Examination of Dairy Products (APHA)[9]
SMWW Standard Methods for the Examination of Water and Wastewater (APHA)[10]
USDA USDA/FSIS Microbiology Laboratory Guidebook[11]
USP The United States Pharmacopeia[12]

For many prepared media, icons are provided denoting the listing of these media in selected publications describing microbiological procedures:

BS12 Bailey & Scott's Diagnostic Microbiology, 12th ed.[13]
CMPH2 Clinical Microbiology Procedures Handbook (ASM)[14]
MCM9 Manual of Clinical Microbiology, 9th ed. (ASM)[15]
CLSI Clinical and Laboratory Standards Institute[16-18]

Because procedures specified in these publications may differ from one another and from those included in this manual, these publications should be consulted when adherence to specific procedures is preferred or required.

As new information becomes available between printings of this manual, individual product descriptions will be updated and available on our web site at www.bd.com/ds/DifcoBBLManual.

Technical inquiries about BD products should be directed to BD Diagnostics Technical Services in the United States at 800-638-8663 or consult www.bd.com/support/contact/international.asp for your local BD Diagnostics office.

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History
BD Diagnostics – A Tradition of Excellence

Original Difco Laboratories Manufacturing facility.

Difco Laboratories, originally known as Ray Chemical, was founded in 1895. The company produced high quality enzymes, dehydrated tissues and glandular products. Ray Chemical acquired the Digestive Ferments Company, a company that specialized in producing digestive enzymes for use as bacterial culture media ingredients. This merger lead to the preparation of a line of peptones, beginning with **Bacto**™ Peptone, and dehydrated culture media. In 1913, the company moved to Detroit, Michigan, and dropped the Ray Chemical name.

In 1934, the Digestive Ferments Company chose the acronym "Difco" to rename the company. The focus of Difco Laboratories was to develop new and improved bacteriological culture media, many of which were adopted as "standard" formulations in water, dairy, food, pharmaceutical and other industrial microbiology laboratories. Difco Laboratories grew through the acquisition, in 1974, of Lee Laboratories, one of the largest manufacturers of bacteriological antisera. The Paul A. Smith Company, later known as Pasco, which manufactured a semi-automated instrument used for bacterial identification and susceptibility testing, was acquired in 1983.

BD's original microbiology products division, acquired in 1955, was founded in 1935 as a partnership between Theodore J. Carski and Dr. Einar Leifson, employees of the Johns Hopkins Hospital in Baltimore, Maryland. Named the Baltimore Biological Laboratory, the laboratory undertook a study of the preparation of peptones, and it began production of three new culture media: Selenite-F Enrichment, Desoxycholate Agar and Desoxycholate-Citrate Agar. The acronym "BBL" was often used and became the brand name for products offered by the company.

The Baltimore Biological Laboratory received increased impetus from the inventions and encouragement of Dr. John Brewer. Brewer's early pipetting machines were produced by the company, and the Brewer anaerobic jar, the forerunner of the **GasPak**™ jar, made the performance of routine anaerobic bacteriology practical and safe.

New discoveries rapidly followed. Incorporation of the reducing chemical sodium thioglycollate resulted in the introduction of thioglycollate media for the cultivation of anaerobes. Other new formulations were added resulting in the development of a full line of culture media. Many of these media utilize peptones of known derivation, such as **Trypticase**™ Peptone, a pancreatic digest of casein, and **Phytone**™ Peptone, a papaic digest of soybean meal, ingredients which are employed in **Trypticase** Soy Agar, **Trypticase** Soy Broth and many other media.

In 1952, the formulation of the U.S. version of Lowenstein-Jensen Medium was introduced, launching the prepared tubed media line. In 1960, the line of prepared culture media was completed by introducing commercially-prepared plated media.

Over the years, BD's microbiology division grew through a series of mergers and acquisitions. In 1972 and 1979, BD purchased two more Baltimore-based microbiology companies – Hynson Wescott and Dunning (HW&D) and Johnston Laboratories, Inc., respectively. HW&D brought with it the **Macro-Vue**™ RPR, **RUBAscan**™ and **CMVscan**™ card test kits and the **Directigen**™ line of immunomicrobiology systems for the direct non-growth-dependent detection of antigens in patient specimens. Johnston Laboratories was the developer and manufacturer of the **BACTEC**™ line of automated blood culturing and detection systems. The **BACTEC** System, launched in 1968, was the first automated bacterial detection system to appear on the market. In 2009, **BACTEC** celebrated its 40th anniversary with the launch of its tenth generation – the **BD BACTEC FX** Blood Culture System.

The media lines were strengthened by the acquisition in 1987 of GIBCO Laboratories of Madison, Wisconsin. Many specialty media formulations were added to the existing **BBL**™ brand prepared plated and tubed media product lines.

In 1989, when Marion Laboratories decided to divest its Marion Scientific division's microbiology product lines, it selected BD's microbiology division as the new provider of products such as the **Bio-Bag**™ Environmental systems and **Culturette**™ Toxin CD test kit for rapid *Clostridium difficile* testing.

In 1992, the division acquired the worldwide microbiology business of Roche Diagnostic Systems, adding the **SEPTI-CHEK**™ blood culture bottles and **Enterotube**™ and **Oxi/Ferm**™ identification products.

In June 1997, BD announced the acquisition of Difco Laboratories, Inc. The merger of this subsidiary with BD's Microbiology Systems division brought together the leading providers of microbiology products to industrial and clinical microbiology laboratories worldwide, with a combined total of over 170 years of culture media experience. Both businesses comprise BD Diagnostics – Diagnostic Systems, which is headquartered in Sparks, Maryland, near the city of Baltimore.

The dawning of the twenty-first century heralded the arrival of several new products. In 2000, the **BD ProbeTec**™ ET Amplified DNA Assays for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were launched. Utilizing proprietary homogeneous strand displacement amplification (SDA) technology, these assays were the first real-time DNA amplification tests on the market. This accomplishment was followed by the launch of the **BD Phoenix**™ Automated Microbiology System in 2004 – a fully automated system for the rapid identification and antimicrobial susceptibility testing of bacteria. Not to be outdone, between 2000 and 2006 the prepared media product line introduced seven new chromogenic media formulations – **BBL**™ **CHROMagar**™ prepared plated media for *Candida, Salmonella, Staphylococcus aureus, Listeria, E. coli* O157 and MRSA as well as **CHROMagar** Orientation for detection of urinary tract pathogens.

Eager to expand its commitment to the prevention of healthcare-associated infections (HAIs), in 2006 BD acquired GeneOhm Sciences, Inc., a pioneer in the development of molecular diagnostics for methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* (Cdiff). The **BD GeneOhm**™ MRSA and **BD GeneOhm** Cdiff assays produce results in hours not days, allowing these infections to be controlled before outbreaks occur.

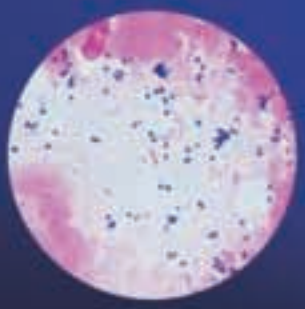
Finally, 2009 will mark the opening of a new, dedicated AF^{2}™ (Animal-Free/Antibiotic-Free) Facility for the cGMP production of cell culture media and supplements. This new plant will manufacture products that are controlled for animal-origin component raw materials to the tertiary level, the most stringent level of control available. The AF^{2} Facility will provide a new standard for safety and quality for cell culture media that significantly reduces current risks associated with mixed-use plants.

BD Diagnostics offers a total spectrum of microbiology laboratory products from dehydrated culture media to fully automated instrumentation for the rapid identification of clinically relevant bacteria. BD Diagnostics continues to focus on the missions and needs of both industrial and clinical microbiology laboratories.

The businesses that now constitute BD Diagnostics were founded by entrepreneurs whose ideas, diligence and foresight have contributed to the creation of BD as one of the world's leaders in the health care field. Through its products and services, BD is committed to "helping all people live healthy lives."

Section I

Monographs



History of Microbiology and Culture Media

The science of microbiology evolved from a series of significant discoveries. The Dutch microscopist, Anton van Leeuwenhoek, was the first to observe bacteria while examining different water sources. This observation was published in 1676 by the Royal Society in London. Anton van Leeuwenhoek was also the first to describe the parasite known today as *Giardia lamblia*. In 1667, the discovery of filamentous fungi was described by Robert Hooke.

After microorganisms were visually observed, their growth or reproduction created a major controversy. The conflict was over the spontaneous generation theory, the idea that microorganisms will grow spontaneously. This controversy continued for years until Louis Pasteur's renowned research. Pasteur realized that the theory of spontaneous generation must be refuted for the science of microbiology to advance. The controversy remained even after Pasteur's successful experiment using heat-sterilized infusions.

Two important developments were required for the science of microbiology to evolve. The first was a sophisticated microscope; the second was a method for culturing microorganisms. Compound microscopes were developed in Germany at the end of the sixteenth century but it was not until the early nineteenth century that achromatic lenses were developed, allowing the light in the microscope to be focused.

In 1719, Leeuwenhoek was the first to attempt differentiation of bacteria by using naturally colored agents such as beet juice. In 1877, Robert Koch used methylene blue to stain bacteria. By 1882, Robert Koch succeeded in staining the tubercle bacillus with methylene blue. This landmark discovery was performed by using heat to penetrate the stain into the organism. Two years later Hans Christian Gram, a Danish pathologist, developed the Gram stain. The Gram stain is still widely used in the differentiation of gram-positive and gram-negative bacteria.

In 1860, Pasteur was the first to use a culture medium for growing bacteria in the laboratory. This medium consisted of yeast ash, sugar and ammonium salts. In 1881, W. Hesse used his wife's agar (considered an exotic food) as a solidifying agent for bacterial growth.

The study of fungi and parasites lagged behind other microorganisms. In 1839, ringworm was the first human disease found to be caused by fungi, followed closely by the recognition of *Candida albicans* as the cause of thrush. It was not until 1910 that Sabouraud introduced a medium that would support the growth of pathogenic fungi. The interest of scientists in studying fungi was often related to crop protection. There continues to be a close connection between mycology and botany today.

Early years at Difco Laboratories.

During the period 1857-1878, both Louis Pasteur and Joseph Lister published significant papers on their extensive studies on fermentation.

By 1887, a simple device called the Petri dish revolutionized microbiology. With the invention of the Petri dish, the focus turned to culture media formulations. With all the research being performed, scientists began to replace gelatin with agar because it was resistant to microbial digestion and liquefaction.

The study of immunity began after the discovery of the tubercle bacillus by Robert Koch. With this acclaimed discovery, the involvement of bacteria as agents of disease became evident. The first rational attempts to produce artificial active immunity were by Pasteur in 1880 during his work with cholera.

Antibiotics had a dramatic beginning with the famous discovery of penicillin by Alexander Fleming in 1928. Fleming observed that a mold had contaminated a culture of staphylococci and produced a substance inhibiting growth of the bacteria. It was not until the late 1930s that scientists could purify penicillin and demonstrate its antibacterial effects. Commercial production of penicillin began as a combined wartime project between the United States and England. This project was the beginning of the fermentation industry and biotechnology.

Around 1930, certain growth factors, including factors X and V, were shown to be important in bacterial nutrition. In the early 1950s, most of the vitamins were also characterized as co-enzymes. This detailed information lead scientists to develop an understanding of biochemical pathways.

A "booming" development of microbiology began after World War II. Molecular biology, biotechnology and the study of genetics were fields of extraordinary growth. By 1941, the study of microbiology and genetics came together when *Neurospora crassa*, a red bread mold, was used to study microbial physiology. The study of bacterial genetics moved dramatically forward during the 1940s following the discovery of antibiotic resistance. The birth of molecular biology began in 1953 after the publication by Watson and Crick of the structure of DNA.

In 1953, viruses were defined by Luria as "submicroscopic entities, capable of being introduced into specific living cells and of reproducing inside such cells only." The work of John Enders on culturing viruses lead to the development of vaccines. Enders demonstrated that a virus could be grown in chick embryos and would lose its ability to cause disease after successive generations. Using this technique, Salk developed the polio vaccine.

One organism that has made a great contribution to molecular biology is *Escherichia coli*. In 1973, Herbert Boyer and Stanley Cohen produced recombinant DNA through plasmid transformation. The researchers found that the foreign gene not only survived, but copied the genetic material. This study and other similar studies started a biotechnology revolution that has gained momentum over the years.

In the 1980s, instrumentation entered the microbiology laboratory. Manual procedures could be replaced by fully automated instruments for bacterial identification, susceptibility testing and blood culture procedures. Immunoassays and probe technologies broadened the capabilities of the microbiologist.

Significant advances continued in the 1990s. The use of chromogenic substrates in culture media was shown to enhance microbial identification capabilities directly from the culture medium. In 1995, J. Craig Venter, Claire Fraser and Hamilton Smith published the DNA sequence of the first free-living organism, *Haemophilus influenzae*.

With rapid advances in technologies and instrumentation, the basic culture media and ingredients listed in this Manual remain some of the most reliable and cost effective tools in microbiology today.

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Microorganism Growth Requirements

Cultivation of microorganisms depends on a number of important factors:

- Proper nutrients must be available.
- Oxygen or other gases must be available, as required.
- Moisture is necessary.
- The medium must have an appropriate pH.
- Proper temperature relations must prevail.
- The medium must be free of interfering bioburden.
- Contamination must be prevented.

A satisfactory microbiological culture medium must contain available sources of:

- Carbon
- Nitrogen
- Inorganic phosphate and sulfur
- Trace metals
- Water
- Vitamins

These were originally supplied in the form of meat infusion. Beef or yeast extracts frequently replace meat infusion in culture media. The addition of peptones, which are digests of proteins, provides readily available sources of nitrogen and carbon.

The pH of the culture medium is important for microorganism growth. Temperature is another important parameter: mesophilic bacteria and fungi have optimal growth at temperatures of 25-40°C; thermophilic ("heat loving") organisms grow only at temperatures greater than 45°C; psychrophilic ("cold loving") organisms require temperatures below 20°C. Human pathogenic organisms are generally mesophiles.

Common Media Constituents

Media formulations are developed on the ability of bacteria to use media components.

CONSTITUENTS	SOURCE
Amino-Nitrogen	Peptone, protein hydrolysate, infusions and extracts
Growth Factors	Blood, serum, yeast extract or vitamins, NAD
Energy Sources	Sugar, alcohols and carbohydrates
Buffer Salts	Phosphates, acetates and citrates
Mineral Salts and Metals	Phosphate, sulfate, magnesium, calcium, iron
Selective Agents	Chemicals, antimicrobials and dyes
Indicator Dyes	Phenol red, neutral red
Gelling agents	Agar, gelatin, alginate, silica gel

Media Ingredients

Purified water is recommended for use in the preparation of culture media. As defined by the *United States Pharmacopeia*, Purified Water is water obtained by a suitable process. It is prepared from water complying with the U.S. Environmental Protection Agency National Primary Drinking Water Regulations or comparable regulations of the European Union or Japan. It contains no added substance.[1]

Peptone, protein hydrolysates, infusions and extracts are the major sources of nitrogen and vitamins in culture media. Peptones are water-soluble ingredients derived from proteins by hydrolysis or digestion of the source material; e.g., meat, milk.

Carbohydrates are employed in culture media as energy sources and may be used for differentiating genera and identifying species.

Buffers maintain the pH of culture media.

Selective Agents include bile salts, dyes and antimicrobial agents. Bile salts and desoxycholate are selective for the isolation of gram-negative microorganisms, inhibiting gram-positive cocci.

Dyes and indicators are essential in the preparation of differential and selective culture media. In these formulations, dyes act as bacteriostatic agents or indicators of changes in acidity or alkalinity of the substrate.

Antimicrobial agents are used in media to inhibit the growth of bacteria, yeasts and fungi.

Solidifying agents, including agar, gelatin and albumin, can be added to a liquid medium in order to change the consistency to a solid or semisolid state.

Chromogens and fluorogens are substrates incorporated into culture media to allow organism differentiation and identification. When these substrates are degraded by specific enzymes, they release differently colored or fluorescent compounds. An example of the latter is 4-methylumbelliferyl-β-D-glucuronide or MUG.

Environmental Factors in Culture Media

Atmosphere

Most bacteria are capable of growth under ordinary conditions of oxygen tension. Obligate aerobes require the free admission of oxygen, while anaerobes grow only in the absence of atmospheric oxygen. Between these two groups are the microaerophiles, which develop best under partial anaerobic conditions, and the facultative anaerobes, which are capable of growing in the presence or absence of oxygen. Anaerobic conditions for growth of microorganisms are obtained in a number of ways:

- Addition of small amounts of agar to liquid media
- Addition of fresh tissue to the medium
- Addition of a reducing substance to the medium; e.g., sodium thioglycollate, thioglycollic acid and L-cystine
- Displacement of the air by hydrogen or nitrogen
- Absorption of the oxygen by chemicals
- Inoculation into the deep layers of solid media or under a layer of oil in liquid media

Many microorganisms require an environment of 5-10% CO_2. Levels greater than 10% are often inhibitory due to a decrease in pH as carbonic acid forms. Culture media vary in their susceptibility to form toxic oxidation products if exposed to light and air.

Water Activity

Proper moisture conditions are necessary for continued luxuriant growth of microorganisms. Organisms require an aqueous environment and must have "free" water. "Free" water is not bound in complex structure and is necessary for transfer of nutrients and toxic waste products. Evaporation during incubation or storage results in loss of "free" water and reduction of colony size or total inhibition of organism growth.

Protective Agents and Growth Factors

Calcium carbonate, soluble starch and charcoal are examples of protective agents used in culture media to neutralize and absorb toxic metabolites produced by bacterial growth.

Nicotinamide adenine dinucleotide, NAD (V factor) and hemin (X factor) are growth factors required by certain bacteria; e.g., *Haemophilus* species, and for enhanced growth of *Neisseria* species.

Surfactants, including polysorbate 80, lower the interfacial tension around bacteria suspended in the medium. This activity permits more rapid entry of desired compounds into the bacterial cell and can increase bacterial growth.

Reference

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Functional Types of Culture Media

The composition of a particular culture medium formulation determines the classification of the medium as general-purpose, enriched, selective or differential or a combination of these types. A general-purpose medium is one that supports the growth of a wide variety of microorganism types and lacks inhibitory properties. Such a medium may be either nonenriched or enriched with an additive, usually animal blood, in order to cultivate highly fastidious microbial species. Examples of such media

include **Trypticase**™ Soy Agar (nonenriched) and **Trypticase** Soy Agar with 5% Sheep Blood (enriched).

A selective medium favors the recovery of specific types or genera of microorganisms and inhibits other members of a mixed flora. Selectivity is usually achieved with a chemical agent, dye or antibiotic. Group A Selective Strep Agar with 5% Sheep Blood (**ssA**™) is an example of a selective medium as it preferentially isolates group A streptococci from throat cultures due to the inclusion of a combination of selective agents. Culture media differ in their degree of selectivity, and the highly selective ones may inhibit some strains of the organisms the recovery of which is desirable. For example, when attempting isolation of enterics, several media possessing varying degrees of selectivity should be utilized.

A differential medium is one which possesses certain ingredients that enable presumptive identification of a specific genus or species either from a pure or mixed culture. Identification is usually based on the organism's appearance; i.e., colony color or the presence of a precipitate. For example, TSI Agar (Triple Sugar Iron Agar) is a differential medium for gram-negative enteric organisms on the basis of their ability to ferment dextrose, lactose and sucrose and to produce sulfides.

Many culture media formulations combine the properties of selectivity and differentiation due to their inclusion of a variety of chemicals. Chromogenic media fall into this category. In addition to selective agents, these unique formulations contain chromogenic substrates which release differently colored compounds upon degradation by specific enzymes. This enzymatic reaction produces distinct colony colors that allows for easy differentiation and identification. For example, on **BBL**™ **CHROMagar**™ Staph aureus medium, *Staphylococcus aureus* produces mauve-colored colonies while most gram-positive organisms, if not inhibited, produce white, blue, green or blue-green (aqua) colonies. Gram-negative organisms and yeasts are partially to completely suppressed.

The isolation of microorganisms from clinical materials frequently requires the use of enriched broth media in addition to the selective, differential and nonselective plated media normally used for primary isolation. The use of liquid "back up" media reduces the possibility of completely missing an etiological agent that is present in low numbers, slow growing on plated media, susceptible to selective agents, or sensitive to unfavorable incubation conditions.

Culture Media Ingredients – Agars

History

Agar was discovered in 1658 by Minora Tarazaemon in Japan.[1] According to legend, this Japanese innkeeper threw surplus seaweed soup into the winter night and noticed it later transformed into a gel by the night's freezing and the day's warmth.[2] In 1882, Koch was the first to use agar in microbiology.[3,4] Walter Hesse, a country doctor from Saxony, introduced Koch to this powerful gelling agent.[5] Hesse had learned about agar from his wife, Fanny Hesse, whose family had contact with the Dutch East Indies where agar was being used for jellies and jams.[3,5,6]

By the early 1900s, agar became the gelling agent of choice instead of gelatin. Agar was found more suitable because it remained solid at the temperatures required for growth of human pathogens and was resistant to breakdown by bacterial enzymes.

Production of agar in the United States was started just before the beginning of World War II as a strategic material.[5] In the 1940s, bacteriological-grade agar manufactured by the American Agar Company of San Diego, California, served as reference agar for the evaluation of the characteristics of other culture media components, such as peptones.[5]

Characteristics

Agar is a phycocolloid, water-soluble polysaccharide, extracted from a group of red-purple marine algae (Class *Rhodophyceae*) including *Gelidium* and *Gracilaria*.[7] These red-purple marine algae are widely distributed throughout the world in temperate

Agar is derived from a group of red-purple marine algae as pictured above.

zones. *Gelidium* is the preferred source of agar. The most important properties of agar are:[5]

- Good transparency in solid and gel forms to allow identification of colony type
- Consistent lot-to-lot gel strength that is sufficient to withstand the rigors of streaking but not so stiff that it affects diffusion characteristics
- Consistent gelling (32-40°C) and melting (approximately 85°C) temperatures, a property known as hysteresis
- Essential freedom from metabolically useful chemicals such as peptides, proteins and fermentable hydrocarbons

- Low and regular content of electronegative groups that could cause differences in diffusion of electropositive molecules (e.g., antibiotics, nutrients)
- Freedom from toxic substances (bacterial inhibitors)
- Freedom from hemolytic substances that might interfere with normal hemolytic reactions in culture media
- Freedom from contamination by thermophilic spores

Agars are normally used in final concentrations of 1-2% for solidifying culture media. Smaller quantities of agar (0.05-0.5%) are used in culture media for motility studies (0.5% w/v) and growth of anaerobes (0.1%) and microaerophiles.[2]

The Manufacturing Process

The finest *Gelidium* marine algae from world sources is selected. Through a variety of processes, the agar is extracted from the *Gelidium*, resulting in a liquid agar that is purified. The liquid agar is first gelled, causing the soluble and suspended contaminants to be trapped, then washed from the agar, eliminating the contaminants. Detailed manufacturing methods are described in references.[2,5]

Product Applications

For specific product applications, refer to the product description for "Agars."

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Culture Media Ingredients – Peptones and Hydrolysates

History

Peptones were originally described in 1880 by Naegeli.[1] With the rich amino acid and nitrogen compounds readily utilized by bacteria, peptones soon became one of the most important constituents of culture media.

Beginning in 1895, Difco Laboratories produced high quality enzymes, dehydrated tissues and glandular products to aid in the digestion process. The knowledge gained from processing animal tissues, purifying enzymes and performing dehydration procedures allowed a smooth transition to the preparation of dehydrated culture media, in addition to its peptones. Meat and other protein digests were developed to stimulate growth of bacteria and fungi. Extensive research led to the development of **Bacto**™ Peptone, which was introduced in 1914.

Since then, **Bacto** Peptone has been recognized as the premium quality standard for all other peptones. Building on this knowledge base, Difco Laboratories continued to develop more peptones to add to the **Bacto** line of products. **Bacto** Proteose Peptone, **Bacto** Proteose Peptone No. 2, and **Bacto** Proteose Peptone No. 3 were created from the accumulated information that no single peptone was the most suitable nitrogen source for growing fastidious bacteria and supplementing mammalian cell culture. Today, many cell culture procedures, in addition to microbial cultures, call for the addition of a peptone to enhance yield.

In 1935, the Baltimore Biological Laboratory (BBL) was founded by Theodore J. Carski and Dr. Einar Leifson, employees of the Johns Hopkins Hospital. The laboratory undertook a study of the preparation of peptones. The acronym "**BBL**" was often used and became the brand name for products offered by the company. New formulations were added, resulting in the development of a full line of culture media. Many of these media utilize peptones of known derivation, such as **Trypticase**™ Peptone, a pancreatic digest of casein, and **Phytone**™ Peptone, a papaic digest of soybean meal.

In 1955, BD acquired BBL and used its expertise to continually advance the clinical market with prepared media and diagnostic tools. In 1997, BD acquired Difco Laboratories and merged Difco Laboratories and BBL Microbiology Systems into one BD division that provides customers with media, peptones/hydrolysates and extracts. Today, the Difco Laboratories facility in Detroit and the BD facility in Baltimore continue to manufacture many of the peptones and hydrolysates offered separately as **Bacto**, **Difco** and **BBL** brands or used in a variety of **Difco** or **BBL** dehydrated culture media formulations.

In late 2009, BD will open a new, dedicated AF²™ (Animal-Free/Antibiotic-Free) Facility for the cGMP production of cell culture media and supplements. The plant, located in Miami, will manufacture products that are controlled for animal-origin component raw materials to the tertiary level, the most stringent level of control available. The AF² Facility will provide a new standard for safety and quality for cell culture media that significantly reduces current risks associated with mixed-use plants.

BD remains committed to producing the highest quality peptones that our customers rely on and expect.

Biochemical Processes

Proteins are molecules essential to the structure and function of all living organisms. They are made up of chains of any number of amino acids linked by peptide bonds and folded in a variety of complex structures. Proteins may be broken down into amino acids and peptides by hydrolysis, using strong acids, bases or proteolytic enzymes, in order to provide nutrients in forms that cells may easily utilize. Protein hydrolysates, also called peptones, are the result of the hydrolysis process on protein material.

The unique characteristics of each BD peptone product depends on the quality and source of the protein starting material, the quality and source of the enzyme, and the method of hydrolysis used to make the peptone. The starting materials for peptones vary from animal to vegetable. Protein sources include meat, casein and whey (milk proteins), gelatin, soybean, yeast and grains.[2] Enzyme sources include animal organs (pancreatin and pepsin), papaya (papain), fig (ficin), pineapple (bromelain) and microbes.[2]

Protein Hydrolysis

Acid hydrolysis is a harsh process, usually carried out at high temperatures, which attacks all peptide bonds in the protein substrate, destroying some of the individual amino acids liberated. Tryptophan is usually totally lost in an acid hydrolysis. Cystine, serine and threonine are partially broken down and asparagine and glutamine are converted to their acidic forms. Vitamins are mostly destroyed by acid hydrolysis. Salts may be formed during neutralization of an acid hydrolysis, resulting in a product with high salt content.

Proteolytic enzymes hydrolyze proteins more gently than acids, do not require the high temperature and usually require specific peptide bonds. The material that results from a proteolytic digestion is a mixture of amino acids and polypeptides of varying lengths. The enzyme pepsin will cut an amino acid chain where there is a phenylalanine or leucine bond.[3] Papain will cut the chain adjacent to arginine, lysine and phenylalanine,[3] and pancreatin shows activity at arginine, lysine, tyrosine, tryptophan, phenylalanine and leucine bonds.[3]

Microbial proteases, proteolytic enzymes secreted by microorganisms, are becoming more widely used in peptone production. Proteases from bacterial, algal, fungal and yeast sources cover a wide variety of enzyme activities, can be produced in large scale and usually require only simple purification.[4]

Peptone Manufacture

Most peptones are manufactured similarly, with steps for hydrolysis and downstream processing. The basic steps of peptone manufacture include: hydrolysis/digestion, centrifugation, filtration, concentration and drying. Protein and demineralized water are combined to form a thick suspension of protein material in large-capacity digestion vessels, which are stirred continuously throughout the digestion process. The protein suspension is then adjusted to the optimum pH for the specific enzyme chosen for the hydrolysis. For example, pepsin is most effective at pH 2.0 and trypsin shows maximum activity at pH 8.5.[2] Enzyme is added when the pH and temperature are optimal. The amount of enzyme necessary, time for digestion, and control of pH and temperature are dependent on the desired degree of hydrolysis.

Once the predetermined degree of protein digestion is achieved, the enzymatic activity must be halted; the suspension is either heated to inactivate the enzymes or neutralized to inactivate acids or bases. The protein slurry is then centrifuged and/or filtered to remove the insoluble materials and to clarify and concentrate the product. Vacuum-evaporation may be used for rapid concentration. The peptone syrup, which contains approximately 67% solids, may undergo further processing for pH adjustment, pasteurization, and/or filtration. The final drying step of the process further concentrates the peptone by spray-drying or by pan-drying in vacuum ovens, which readies the material for packaging.

Ultrafiltration

Ultrafiltration (UF) is a membrane filtration process used to separate or concentrate constituents of protein solutions based on molecular weight. BD offers several peptone and yeast extract products that are ultrafiltered using a 10k Dalton Molecular Weight Cut Off (MWCO) membrane. The result of using the 10k Da MWCO is a retentate containing molecules over 10k Da MW, which may include fats, larger MW polypeptides and proteins, and a permeate that contains salts, sugars, peptides, smaller polypeptides and other compounds of less than 10k Da MW.

In peptone manufacture, ultrafiltration is used to create a product that is low in endotoxin, the toxin-containing lipopolysaccharide part of the cell wall shed from viable gram-negative bacteria and released when gram-negative bacteria die. Endotoxins will cause illness in humans, so they are considered contaminants that must be avoided or minimized in the preparation of pharmaceutical products. The ultrafiltration step takes place before drying in the peptone manufacturing process.

Meat Peptones

Meat peptones are proteins from animal sources that have been hydrolyzed, or broken down into amino acids and peptides, to provide nitrogen for microorganisms. Meat peptones can be tailored to specific nutritive needs of microorganisms by controlling the quality and origin of the protein, the quality and source of the enzyme used to digest the protein, and the methods used for hydrolysis, concentration and drying the peptone. Sources of animal protein include meat from muscular tissue or offal (waste parts, entrails) and gelatin. Muscular tissue and offal are utilized fresh, frozen or dried. Gelatin is extracted by boiling collagen, the fibrous protein found in connective tissue, bone and cartilage.

A variety of proteolytic enzymes, or proteases, may be used to accomplish enzymatic hydrolysis of animal protein. Pepsin and trypsin are widely used for animal peptone manufacture. Pepsin is isolated from porcine or other animal stomach. Trypsin, along with chymotrypsin, carboxypeptidase A, carboxypeptidase B, and elastase, are enzymes isolated from animal pancreas.

Casein Peptones

Casein and whey peptones are hydrolysates of bovine milk proteins. Milk is a complex material, consisting of water, lactose, lipids, salts and proteins.

Casein (80%) and whey (20%) are the fundamental protein components in milk. After the cream, or fat, has been removed from bovine milk, hydrochloric or sulfuric acid is added in order to precipitate out casein, the insoluble portion.[5,6] The casein recovered is known as acid casein and is insoluble in water. Generally, the acid casein is dissolved in a suitable hydroxide such as NaOH, to make it soluble in water. The resulting sodium caseinate is then used as the basis for hydrolyzed caseins. Sodium caseinate typically consists of 87% to 90% protein.[7] Casein, which can make up to 3% of the total components in bovine milk, is one of the most nutritive of the milk proteins, as it contains all of the common amino acids and is rich in the essential ones.

The soluble supernatant material separated from milk after casein precipitates is whey, also called milk plasma. Whey contains the lactalbumin and lactoglobulin proteins and is a by-product of casein (and cheese) production. Whey protein concentrates and isolates are recovered using various separation technologies such as ion exchange and filtration; lactalbumin is recovered by heat denaturing and then separation.[5] Whey peptones are manufactured using the process of enzymatic hydrolysis on the proteins isolated from whey. The whey peptones contain free amino acids and peptides, as well as carbohydrates, vitamins and minerals.

Casein peptones are manufactured by either acid or enzymatic hydrolysis. Many acids can be utilized in the acid hydrolysis of casein, but hydrochloric acid is most commonly used in the process. This process leads to complete hydrolysis of the casein to amino acids and other compounds of relative chemical simplicity.

The manufacturing process for an enzymatic hydrolysis of casein is not as destructive as an acid hydrolysis. The casein is not broken down as completely into its constituent components. In many cases, this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids. Enzymes from the pancreas are utilized to manufacture these peptones. While the pancreas contains a battery of enzymes from the protease, lipase and amylase groups, it is the proteases that are used in the hydrolysis of casein. These proteases only have the ability to digest proteins into peptides; they cannot break the protein down into its component amino acids. As a result, pancreatic digests of casein, as opposed to acid hydrolysates of casein, produce peptones that contain greater amounts of peptides.

Soy Peptones

Soy peptones are all enzymatic digests of soy flour. Soy contains several heat labile protease inhibitors.[8] The most common way of eliminating these factors is to heat or toast the defatted soy beans in a processing plant under controlled conditions. Soy flour, the principle substrate in a soy peptone, is rich in high-quality protein, carbohydrates, calcium and B vitamins.[9] The enzymes used in the digestion of the soy flour are typically from animal-free sources or from microorganisms that have been grown in animal-free media.

Yeast Products

Yeast extract is defined in the *USP* as "a water-soluble, peptone-like derivative of yeast cells (*Saccharomyces*)."[10] Yeast extract is readily available in the U.S. as a spray-dried powder. In Europe, pharmaceutical companies use it as a liquid or paste, as well as in the powdered form.

Yeast extract is used by the health food industry as an inexpensive source for many of their vitamins, and has long been recognized as a major source of B-complex vitamins. Yeast extract, as a substrate in a media formulation, supplies not only vitamins, but also proteins, carbohydrates and some micronutrients.

There are many kinds of yeast extract. The two principle sources of yeast extract are "brewer's" yeast and "baker's" yeast. Brewer's yeast is a by-product from the brewing industry. It requires de-bittering (removal of hop resins) before it is suitable for fermentation use.[2] A wide variety of strains and growth processes have been used in the manufacture of the brewer's yeast, thus precluding any consistency of the final product.

Baker's yeast (*Saccharomyces cerevisiae*) is defined as a primary yeast because the yeast is grown for the specific purpose of being used as a substrate in a bioprocess or as a food product/flavoring. Manufacture of baker's yeast is a reproducible and controlled process. The yeast organism is grown on a molasses-based medium optimized for the specific yeast.[11] Commercial yeast fermentations are always fed-batch type fermentations lasting from 12-20 hours.[12] Commercial baker's yeast manufacturers have found that the more highly aerated a culture, the higher the final product yield.[12]

The process of manufacturing baker's yeast extract is unique compared to the manufacture of other peptones. Yeast extract is an autolysate. Cell hydrolysis is performed by the endogenous enzymes of the *Saccharomyces* organism. Autolysis is usually begun by either a controlled temperature shock or, for the food industry, an osmotic shock, which causes the yeast cells to expire. The temperature shock is not high enough to inactivate the proteases of the yeast cell, which proceed to degrade the cell. Autolysis can proceed from 10 to 60 hours.

After autolysis, soluble material is separated from insoluble by means of centrifugation and several filtration steps.[12] The final filtration product is concentrated and then spray dried, or can be left in the concentrated paste form, which contains approximately 60-80% solids.

Temperature, pH, addition of other enzymes, type of medium substrate for the growth of the *Saccharomyces* and duration of autolysis are all variations that create the large variety of yeast extracts available.

Peptone Performance

The raw materials and manufacturing conditions for protein hydrolysis are controlled to produce consistent peptone products. Ingredients used for peptone manufacture, including the protein, agent of hydrolysis, and any buffering agents used, are

selected based on specific purity and quality standards. The conditions of the hydrolysis, such as the amount of enzyme used, the time for digestion, and the pH and temperature at which hydrolysis is conducted, determine the degree of hydrolysis and the quality of the hydrolysate. Therefore, these conditions must be carefully controlled throughout the manufacturing process. Purification, concentration and drying steps are carefully regulated due to their bearing on the characteristics of a peptone. Finally, each batch of protein hydrolysate is tested for an array of physical, chemical, analytical and growth support tests to ensure product quality and lot-to-lot consistency.

Peptones/hydrolysates are available as original premium **Bacto**™ formulations, or as **Difco**™, **BBL**™ or **BiTek**™ brands. The **BiTek** brand was developed for production-grade products where a premium product is not required.

Applications

Cell Culture

In the biopharmaceutical industry, the requirement for high-performance animal-free processes has prompted a greater focus on media and process optimization.[13] Serum supplementation has traditionally been used to compensate for base media deficiencies. However, the regulatory issues and high costs associated with using serum have led to a widespread effort to find animal free or chemically defined alternatives. While chemically defined media are ideal, they often do not meet the expected production goals.

Through additional work, it has been observed that peptone supplementation, when appropriately applied, can exceed the performance of serum while meeting stringent regulatory requirements. Additionally, downstream processing requirements are greatly reduced due to the lack of contaminating serum proteins, thereby reducing processing time and costs. In order to achieve this type of success, a complete process optimization must occur where the base media, peptone supplementation, and feed strategy are empirically determined through the use of a methodical optimization strategy.[14]

The benefits of peptone supplementation in cell culture applications have been well documented for many years. Due to the complex composition of peptones, they provide a wide range of benefits to the cells. In some cases, peptides of various lengths have resulted in increased cell performance.[15] Others have benefited from free amino acids and other low molecular weight nutrients.[16] Since the nutritional requirements for each cell line are different, it is important to identify a peptone that will meet the unique requirements of a particular cell line. Blends of peptones should also be considered, as synergistic effects have been observed in some processes when multiple peptones were used.[17] Significantly higher antibody yields can be achieved with the identification of a peptone that meets the specific requirements of the cell line.

Determining how to apply the peptone is essential to achieving the increased performance. While some processes require that peptone is present from the beginning of the run, others perform best when the peptone is added as a feed later in the process. In some cases, optimal performance is achieved when the process begins with one peptone then another is added as a feed later in the production run.

BD AutoNutrient™ Media Design Service (AMDS)

Performing thorough optimizations can require significant time and resources, so the decision is often made to eliminate many potentially critical design points. To address this need and ensure the identification of the optimal media formulation that meets production goals, BD offers the **AutoNutrient**™ Media Design Service (AMDS). The BD team of dedicated, experienced scientists works with each customer in a highly collaborative process to develop a media formulation that satisfies the requirements. Through the AMDS program, BD offers a library of 45 diverse, chemically defined media, as well as a number of peptones designed specifically for the biopharmaceutical industry. AMDS partners with each customer from initial screens through final scale up to ensure an optimized process at each step.

Fermentation

Fermentation media formulations are of two types: defined and complex. Defined media are made by the addition of chemically defined ingredients to water for injection (WFI) or distilled water. Complex media are made with peptone digests or extracts of plant or animal origin.[18]

The advantages of chemically defined media are greater reproducibility, cleaner downstream processing steps and simplicity in the analysis of the end product. The disadvantages are lower yields and greater expense, especially if the list of media components include growth factors and vitamins.[19] The advantages of complex media are that they are relatively inexpensive, they support a wide variety of growth from a large group of microorganisms, they promote growth of the more fastidious organisms that will not grow on a chemically-defined medium, they stimulate toxin production and they routinely produce higher yields than many defined media. The disadvantages of complex media are that the downstream processing may be more difficult and reproducibility can sometimes be compromised. When developing a new medium formulation, care should be taken in choosing the peptones for the new formulation. Individual experimentation with a variety of peptones is suggested to select the optimum peptone or combination of peptones.

With the continuing emergence of new confirmed cases of BSE/TSE, a prime directive for the development of new fermentation products has been to either source raw materials from a country free from BSEs or reformulate the media using animal-free components.[20] BD began offering animal free alternatives to classical media formulations in 1997. In 1998, **Select APS**™ (alternative protein source) products were introduced. These non-animal formulations provide growth support characteristics comparable, and in some cases superior, to classical animal formulations.

Culture Media Ingredients

Beef Extract
Bacto™ Beef Extract, Desiccated
Beef Extract Powder

The beef extract products are replacements for aqueous infusions of meat. Beef extract is not exposed to the harsh treatment used for protein hydrolysis, so it can provide some of the nutrients lost during peptone manufacture.[19] Beef Extract is a mixture of peptides and amino acids, nucleotide fractions, organic acids, minerals and some vitamins. Its function is to complement the nutritive properties of peptone by contributing minerals, phosphates, energy sources and those essential factors missing from peptone.[2]

Beef extract is usually employed in concentrations of 0.3 to 1.0% in culture media. Beef Extract is in a paste form. **Bacto** Beef Extract, Desiccated, the dried form of Beef Extract, was developed to provide a product for ease of use in handling. These two products are used in a one for one substitution. Beef Extract Powder is a meat extract dried to a powdered form.

Bacto™ Beef Heart for Infusion

Bacto Beef Heart for Infusion is prepared from fresh beef heart tissue and is recommended for preparing heart infusion media. It provides nitrogen, amino acids and vitamins in microbiological culture media. **Bacto** Beef Heart for Infusion is processed from large volumes of raw material, retaining all the nutritive and growth stimulating properties of fresh tissue.

Biosate™ Peptone

Biosate Peptone is a mixed hydrolysate of casein and yeast extract. The product provides nitrogen, amino acids and vitamins in microbiological culture media. The combination of pancreatic digest of casein and yeast extract, at a ratio of 65:35, provides nutritional benefits that are not provided by the components alone.

Bacto™ Casamino Acids
Bacto™ Casamino Acids, Technical
Casamino Acids, Vitamin Assay
Acidicase™ Peptone

Bacto Casamino Acids, **Bacto** Casamino Acids, Technical, Casamino Acids, Vitamin Assay and **Acidicase** Peptone are acid hydrolysates of casein. Casein is a milk protein and a rich source of amino acid nitrogen. These products are added to media primarily because of their organic nitrogen and growth factor components. Their inorganic components also play a vital role.[21]

Bacto Casamino Acids is recommended for use with microbiological cultures that require a completely hydrolyzed protein as a nitrogen source. **Bacto** Casamino Acids, Technical is recommended for use in culture media where amino acid mixtures are required for a nitrogen source but the sodium chloride and iron content have not been decreased to the same extent as with Casamino Acids. Casamino Acids, Vitamin Assay is specially treated to markedly reduce or eliminate certain vitamins. It is recommended for use in microbiological assay media and in growth promotion studies. **Acidicase** Peptone is a hydrochloric acid hydrolysate of casein with a high salt content. It is deficient in cystine, because casein contains little cystine, and in tryptophan, which is destroyed by the acid treatment. It is used in vitamin assay media, susceptibility testing and other laboratory media and microbial fermentation where the high salt content will not interfere.

Bacto™ Casitone
Trypticase™ Peptone
Bacto™ Tryptone

Bacto Casitone, **Trypticase** Peptone and **Bacto** Tryptone are pancreatic digests of casein. These products are recommended for preparing media where an enzymatic hydrolyzed casein is desired. The manufacturing process for an enzymatic digest of casein is not as destructive as an acid hydrolysis. Thus, the casein is not broken down as completely into its constituent components. In many cases, this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids. Casein is a rich source of amino nitrogen. These products are used to support the growth of fastidious microorganisms and are suitable for use in fermentation studies. **Trypticase** Peptone is biologically tested for freedom from detectable carbohydrates. Their high tryptophan content makes **Bacto** Casitone, **Trypticase** Peptone and **Bacto** Tryptone valuable for detecting indole production.

Gelysate™ Peptone

Gelysate Peptone is a pancreatic digest of gelatin. Gelatin is extracted from collagen, which is the fibrous protein in bone, cartilage and connective tissue. Gelatin hydrolysate is high in proline residues.[2] **Gelysate** Peptone is deficient in carbohydrates and is characterized by low cystine, methionine and tryptophan content. **Gelysate** Peptone should be used for cultures requiring low carbohydrate, cystine and tryptophan levels in cell culture and bacterial fermentation.

Bacto™ Malt Extract

Bacto Malt Extract is the water-soluble portion of malted barley. The extraction process breaks down the polysaccharides into simple sugars. After the malting process is complete, the extract is prepared from the malted barley by cracking the grain in a mill and then extracting the grain with a warm liquor. The resulting "wort" is filtered and evaporated or dried under vacuum.[2,22] It is used in culture media for the propagation of yeasts and molds because it contains a high concentration of reduced sugars, particularly maltose. This product is generally employed in concentrations of 1-10%. **Bacto** Malt Extract provides carbon, protein and nutrients in culture media.

Bacto™ Neopeptone

Bacto Neopeptone is an enzymatic digest of protein. This product contains a wide variety of peptide sizes in combination with vitamins, nucleotides and minerals. **Bacto** Neopeptone is particularly well suited in supplying the growth requirements of fastidious bacteria. In addition, this peptone is extremely valuable in media for the cultivation of pathogenic fungi.

Bacto™ Peptone

Bacto Peptone is an enzymatic digest of animal protein. **Bacto** Peptone was first introduced in 1914 and became the standard peptone for the preparation of bacteriological culture media. This peptone contains nitrogen in a form that is readily available for bacterial growth. **Bacto** Peptone has a high peptone and amino acids content and only a negligible quantity of proteoses and more complex nitrogenous constituents. **Bacto** Peptone is used as an organic nitrogen source in microbiological culture media for cultivation of a variety of bacteria and fungi and in cell culture media formulations.

Phytone™ Peptone
Select Phytone, UF
Select Soytone
Bacto™ Soytone

Phytone Peptone, Select **Phytone**, UF, Select Soytone, and **Bacto** Soytone are enzymatic digests of soybean meal/flour. The soybean protein in these peptones contains naturally occurring high concentrations of vitamins and carbohydrates. **Phytone** Peptone is an animal-free soy peptone that retains the high vitamin and high carbohydrate content of the soy plant tissue. This product is an excellent plant peptone for the cultivation of fungi and fastidious types of bacteria. It has also been used in cell culture applications due to its high carbohydrate content.

Select **Phytone**, UF is an ultra-filtered peptone that was developed for the tissue culture market. Its nitrogen content combined with the naturally occurring vitamins has demonstrated remarkable growth support with monoclonal antibodies and protein expression. Select Soytone is used in molecular genetics media and cell culture. **Bacto** Soytone is recommended for use in media for the cultivation of a large variety of organisms, including fungi, and microbiological assay media. **Bacto** Soytone utilizes an animal-based enzyme in the digestion of soy flour.

Polypeptone™ Peptone

Polypeptone Peptone is a mixture of peptones made up of equal parts of pancreatic digest of casein and peptic digest of animal tissue. **Polypeptone** Peptone includes the high content of amino acids and small polypeptides characteristic of pancreatic digest of casein and the larger polypeptides characteristic of peptic digest of animal tissue. **Polypeptone** Peptone provides nitrogen, amino acids and vitamins in microbiological culture media.

Bacto™ Proteose Peptone
BiTek™ Proteose Peptone
Bacto™ Proteose Peptone No. 2
Bacto™ Proteose Peptone No. 3
Bacto™ Proteose Peptone No. 4

Bacto Proteose Peptone, **BiTek** Proteose Peptone, **Bacto** Proteose Peptone No. 2, **Bacto** Proteose Peptone No. 3 and **Bacto** Proteose Peptone No. 4 are enzymatic digests of protein. **Bacto** Proteose Peptone, **BiTek** Proteose Peptone, **Bacto** Proteose Peptone No. 2 and **Bacto** Proteose Peptone No. 4 are used in preparing microbiological culture media and in producing bacterial toxins. **Bacto** Proteose Peptone No. 3 is used to culture fastidious microorganisms. **Bacto** Proteose Peptone No. 4 is a spray-dried version of **Bacto** Proteose Peptone. These proteose peptone products provide nitrogen in a form that is readily available for bacterial growth.

Bacto™ TC Lactalbumin Hydrolysate

Bacto TC Lactalbumin Hydrolysate is the enzymatically hydrolyzed protein portion of milk whey. It is a mixture of peptides, amino acids and carbohydrates, simple and complex. It is used for preparing bacterial, insect and mammalian cell culture media.

Bacto™ TC Yeastolate
TC Yeastolate, UF

Bacto TC Yeastolate and TC Yeastolate, UF are animal-free, water-soluble portions of autolyzed yeast of *Saccharomyces cerevisiae*. These products are mixtures of peptides, amino acids, simple and complex carbohydrates, and vitamins. They are used for preparing bacterial, insect and mammalian cell culture media.

Bacto™ Tryptose

Bacto Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process of **Bacto** Tryptose results in assorted peptides of higher molecular weight suitable for long chain amino acid requirements. **Bacto** Tryptose is used for the cultivation of fastidious organisms and for cell culture applications.

Bacto™ Yeast Extract
Yeast Extract, UF
Yeast Extract, LD
Bacto™ Yeast Extract, Technical
Yeast Extract

Bacto Yeast Extract, Yeast Extract, UF, Yeast Extract, LD, **Bacto** Yeast Extract, Technical and Yeast Extract are concentrates of the water-soluble portion of *Saccharomyces cerevisiae* cells that have been autolyzed. These products provide essential water soluble vitamins, amino acids, peptides and carbohydrates in microbiological culture media. Yeast extract is considered a non-animal product and is used extensively for many non-animal formulations of bacterial, fungal, mammalian and insect cell culture.

For additional information on peptones, call BD Technical Services at 800-638-8663 and request a copy of the *BD Bionutrients™ Technical Manual*.

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Media Sterilization

Sterilization is any process or procedure designed to entirely eliminate viable microorganisms from a material or medium. Sterilization should not be confused with disinfection, sanitization, pasteurization or antisepsis which are intended to inactivate microorganisms, but may not kill all microorganisms present. Sterilization can be accomplished by the use of heat, chemicals, radiation or filtration.[1]

Sterilization with Heat[1]

The principal methods of thermal sterilization include 1) moist heat (saturated steam) and 2) dry heat (hot air) sterilization. Heat kills microorganisms by protein denaturation and coagulation. Moist heat has the advantage of being more rapid and requiring lower temperatures than dry heat. Moist heat is the most popular method of culture media sterilization. When used correctly, it is an economical, safe and reliable sterilization method.

Moist Heat Sterilization

Water boils at 100°C, but a higher temperature is required to kill resistant bacterial spores in a reasonable length of time. A temperature of 121°C for 15 minutes is an accepted standard condition for sterilizing up to one liter of culture medium. The definition of "autoclave at 121°C for 15 minutes" refers to the temperature of the contents of the container being held at 121°C for 15 minutes, not to the temperature and time at which the autoclave has been set.[2] The steam pressure of 15 pounds per square inch at this temperature aids in the penetration of the heat into the material being sterilized. If a larger volume is to be sterilized in one container, a longer period should be employed. Many factors can affect sterility assurance, including size and contents of the load and the drying and cooling time. Certain products may decompose at higher temperature and longer cycles. For this reason, it is important that all loads be properly validated.

The basic principles for validation and certification of a sterilizing process are enumerated as follows:[3]

1. Establish that the processing equipment has the capability of operating within the required parameters.
2. Demonstrate that the critical control equipment and instrumentation are capable of operating within the prescribed parameters for the process equipment.
3. Perform replicate cycles representing the required operational range of the equipment and employing actual or simulated product. Demonstrate that the processes have been carried out within the prescribed protocol limits and, finally, that the probability of microbial survival in the replicate processes completed is not greater than the prescribed limits.
4. Monitor the validated process during routine operation. Periodically as needed, requalify and recertify the equipment.
5. Complete the protocols and document steps 1-4, above.

For a complete discussion of process validation, refer to appropriate references.

Ensuring that the temperature is recorded correctly is vital. The temperature must reach all parts of the load and be maintained for the desired length of time. Recording thermometers are employed for the chamber and thermocouples may be buried inside the load.

For best results when sterilizing culture media, plug tubes or flasks of liquids with nonabsorbent cotton or cap loosely. Tubes should be placed in racks or packed loosely in baskets. Flasks should never be more than two-thirds full. It is important to not overload the autoclave chamber and to place contents so that there is a free flow of steam around the contents. After sterilizing liquids, the chamber pressure must be reduced slowly to atmospheric pressure. This allows the liquid to cool below the boiling point at atmospheric pressure before opening the door to prevent the solution from boiling over.

In autoclave operation, all of the air in the chamber must be expelled and replaced by steam; otherwise, "hot spots" and "cold spots" will occur. Pressure-temperature relations of a properly operated autoclave are shown in the table below.

Pressure-Temperature Relations in Autoclave[4] (Figures based on complete replacement of air by steam)

PRESSURE IN POUNDS	TEMPERATURE (°C)	TEMPERATURE (°F)
5	109	228
10	115	240
15	121	250
20	126	259
25	130	267
30	135	275

Over-sterilization or prolonged heating will change the composition of the medium. For example, carbohydrates are known to break down in composition upon overheating. Over-sterilizing media can cause a number of problems, including:

- Incorrect pH
- A decrease in the gelling properties of agar
- The development of a nontypical precipitate
- Carmelization or darkening of the medium
- Loss of nutritive value
- Loss of selective or differential properties

There are certain media (e.g., Hektoen Enteric Agar and Violet Red Bile Agar) that should not be autoclaved. To dissolve these media formulations, heat to boiling to dissolve completely. It is important to follow all label directions for each medium. Media supplements should be sterile and added aseptically to the sterilized medium, usually at 45-55°C.

Dry Heat Sterilization[1]

Dry heat is employed for materials such as metal instruments that could be corroded by moist heat, powders, ointments and dense materials that are not readily penetrated by steam. Because dry heat is effective only at considerably higher temperatures and longer times than moist heat, dry heat sterilization is restricted to those items, unlike culture media, that will withstand higher temperatures. The dry heat time for sterilization is 120 minutes at 160°C.

Chemical Sterilization[1]

Chemical sterilization employs gaseous and liquid sterilants for certain medical and industrial instruments. The gases include ethylene oxide, formaldehyde and beta-propiolactone. The liquid sterilants include glutaraldehyde, hydrogen peroxide, peracetic acid, chlorine dioxide and formaldehyde. Chemical sterilization is not employed in the preparation of culture media due to unfavorable affects upon performance. For a complete discussion of this topic, consult appropriate references.

Radiation Sterilization[1]

Radiation sterilization is an optional treatment for heat-sensitive materials. This includes ultraviolet light and ionizing radiation.

Ultraviolet light is chemically active and causes excitation of atoms within the microbial cell, particularly the nucleic acids, producing lethal mutations. This action stops the organism from reproducing. The range of the ultraviolet spectrum that is microbiocidal is 240-280 nm. There is a great difference in the susceptibility of organisms to ultraviolet radiation; *Aspergillus niger* spores are 10 times more resistant than *Bacillus subtilis* spores, 50 times more resistant than *Staphylococcus aureus* and *Escherichia coli*, and 150 times more resistant than influenza virus.

Because most materials strongly absorb ultraviolet light, it lacks penetrating power and its applications are limited to surface treatments. Much higher energy, 100 to millions of times greater, is generated by ionizing radiations. These include gamma-rays, high energy X-rays and high energy electrons.

Ionizing radiation, unlike ultraviolet rays, penetrates deeply into atoms, causing ionization of the electrons. Ionizing radiation may directly target the DNA in cells or produce active ions and free radicals that react indirectly with DNA.

Gamma radiation is used more often than x-rays or high-energy electrons for purposes of sterilization. Gamma rays are generated by radioactive isotopes, cobalt-60 being the usual source. Gamma radiation requires many hours of exposure for sterilization. Validation of a gamma irradiation procedure includes:[4]

- Establishment of article materials compatibility
- Establishment of product loading pattern and completion of dose mapping in the sterilization container
- Establishment of timer setting
- Demonstration of the delivery of the required sterilization dose

The advantages of sterilization by irradiation include low chemical reactivity, low measurable residues, and few variables to control.[3] Gamma irradiation is used for treating many heat-sensitive products that can also be treated by gaseous sterilization, including medical materials and equipment, pharmaceuticals, biologicals and laboratory equipment. BD utilizes gamma irradiation in the manufacturing of **BBL**™ Sterile Pack media for environmental monitoring of critical environments.

Sterilization by Filtration[1,3]

Filtration is a useful method for sterilizing liquids and gases. Filtration excludes microorganisms rather than destroying them. Two major types of filters may be used, depth filters and membrane filters.

The membrane filter screens out particles, while the depth filter entraps them. Membrane filters depend largely on the size of the pores to determine their screening effectiveness. Electrostatic forces are also important. A membrane filter with an average pore size of 0.8 µm will retain particulate matter as small as 0.05 µm. For removing bacteria, a pore size of 0.2 µm is commonly used. For retention of viruses and mycoplasmas, pore sizes of 0.01-0.1 µm are recommended. Cocci and bacilli range in size from about 0.3 to 1 µm in diameter. Most viruses are 0.02-0.1 µm, with some as large as 0.25 µm.

Rating the pore size of filter membranes is by a nominal rating that reflects the capability of the filter membrane to retain microorganisms of size represented by specified strains. Sterilizing filter membranes are membranes capable of retaining 100% of a culture of 10^7 microorganisms of a strain of *Pseudomonas diminuta* (ATCC™ 19146) per square centimeter of membrane surface under a pressure of not less than 30 psi. These filter membranes are nominally rated 0.22 µm or 0.2 µm. Bacterial filter membranes (also known

as analytical filter membranes), which are capable of retaining only larger microorganisms, are labeled with a nominal rating of 0.45 µm.

Membrane filters are used for the commercial production of a number of pharmaceutical solutions and heat-sensitive injectables. Serum for use in bacterial and viral culture media are often sterilized by filtration, as well as some sugars that are unstable when heated. Membrane filtration is useful in testing pharmaceutical and medical products for sterility.

Sterility Assurance[1]

Sterility Assurance is the calculated probability that a microorganism will survive sterilization. It is measured as the SAL, Sterility Assurance Level, or "degree of sterility". For sterility assurance, *Bacillus stearothermophilus* which contains steam heat-resistant spores is employed with steam sterilization at 121°C.

Testing Sterilizing Agents[1,5]

Sterilization by moist heat (steam), dry heat, ethylene oxide and ionizing radiation is validated using biological indicators. The methods of sterilization and their corresponding indicators are listed below:

STERILIZATION METHOD	BIOLOGICAL INDICATOR
Steam	*Bacillus stearothermophilus*
Dry heat	*Bacillus subtilis* var. *niger*
Ethylene oxide	*Bacillus subtilis* var. *globigii*
Filtration	*Pseudomonas diminuta*

For moist heat sterilization, paper strips treated with chemicals that change color at the required temperature may be used.

The heat-resistant spores of *B. stearothermophilus* are dried on paper treated with nutrient medium and chemicals. After sterilization, the strips are incubated for germination and growth, and a color change indicates whether they have or have not been activated. Spore strips should be used in every sterilization cycle.

Glossary[1,6]

Bioburden is the initial population of living microorganisms in the product or system being considered.

Biocide is a chemical or physical agent intended to produce the death of microorganisms.

Calibration is the demonstration that a measuring device produces results within specified limits of those produced by a reference standard device over an appropriate range of measurements.

Death rate is the rate at which a biocidal agent reduces the number of cells in a microbial population that are capable of reproduction. This is determined by sampling the population initially, during and following the treatment, followed by plate counts of the surviving microorganisms on growth media.

D value stands for decimal reduction time and is the time required in minutes at a specified temperature to produce a 90% reduction in the number of organisms.

Microbial death is the inability of microbial cells to metabolize and reproduce when given favorable conditions for reproduction.

Process validation is establishing documented evidence that a process does what it purports to do.

Sterility Assurance Level is generally accepted when materials are processed in the autoclave and attain a 10^{-6} microbial survivor probability; i.e., assurance of less than one chance in one million that viable microorganisms are present in the sterilized article.[3]

Sterilization process is a treatment process from which the probability of microorganism survival is less than 10^{-6}, or one in a million.

Thermal Death Time and **Thermal-Chemical Death Time** are terms referring to the time required to kill a specified microbial population upon exposure to a thermal or thermal-chemical sterilizing agent under specified conditions. A typical thermal death time value with highly resistant spores is 15 minutes at 121°C for steam sterilization.

References

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Quality Control Organisms

Bacterial Control Strain Source

An integral part of quality control testing includes quality control organisms. The following procedures for preparing quality control organisms were developed by the Clinical and Laboratory Standards Institute for evaluating the performance of certain commercially-prepared microbiological culture media.[1] These procedures may require modification for the preparation and use of control cultures of mycobacteria, yeasts and molds. Microorganisms should be obtained from the American Type Culture Collection (ATCC™) or other commercial sources.

Maintenance/Frozen Stock Cultures

If using commercial stock cultures, follow the manufacturer's recommendations for growth and maintenance.

To prepare frozen stock cultures of *Staphylococcus* species, *Streptococcus* species, *Enterobacteriaceae* and *Pseudomonas aeruginosa*:

1. Reconstitute the stock culture, if necessary.
2. Inoculate multiple plates of a general-purpose medium; e.g., Tryptic/**Trypticase**™ Soy Agar (TSA) or blood agar.
3. Incubate plates for 18-24 hours in an appropriate atmosphere and at the recommended temperature.
4. Check for purity and correct colony morphology.
5. If necessary, verify using biochemical tests.
6. Remove sufficient growth from a confluent area and suspend in 50-100 mL of cryoprotective medium; e.g., Tryptic/**Trypticase** Soy Broth (TSB) with 10-15% glycerol, skim milk or sterile defibrinated sheep blood.
7. If the frozen cultures will be used to inoculate test media, adjust suspension to a 0.5 McFarland standard ($1\text{-}2 \times 10^8$ CFU/mL). For fastidious organisms, adjust to a 1 McFarland.
8. Dispense 0.5-1.0 mL into sterile glass or plastic freezing vials. Prepare enough vials for one year of storage. Assume only one freeze/thaw cycle per vial. Assume at least one fresh culture every four weeks.
9. Store vials at or below –50°C (freezer) for one year. Organisms will keep longer (indefinitely) if stored in an ultra-low-temperature freezer or in a liquid nitrogen tank.

To use a frozen culture:

1. Thaw the vial quickly.
2. Use the culture directly or subculture.
3. Discard any unused cell suspension. Never refreeze cultures for later use.

Working Cultures

1. Inoculate an agar slant or plate with the frozen stock culture and incubate overnight.
2. Store the working culture at 2-8°C or at room temperature for up to four weeks.
3. Check for purity and appropriate colony morphology.
   NOTE: Prepare no more than three serial subcultures from the original "working culture."

Or

Use the frozen culture directly as a working culture.

Maintain anaerobic cultures in Cooked Meat Medium or another suitable anaerobic medium. Alternatively, use frozen anaerobic cultures.

Test Procedure

1. Inoculate an agar plate from the "working culture."
2. Incubate overnight.
3. Suspend 3-5 isolated colonies with typical appearance in a small volume (0.5-1.0 mL) of TSB. Incubate 4-5 hours in an appropriate atmosphere and temperature.
4. Adjust the turbidity to 0.5 McFarland (0.08-0.1 absorbance units at 625 nm). Alternatively, a suspension may be made from an overnight culture and adjusted to a 0.5 McFarland.
5. Perform a plate count of the organism suspension to confirm a colony count of $1\text{-}2 \times 10^8$ CFU/mL.

To Test Cultural Response

When qualifying a new lot of culture medium, always test the new lot of medium in parallel with an approved lot of medium.

Non-Selective Media

Dilute the cell suspension 1:100 in normal saline or purified water. Inoculate each plate with 0.01 mL (10 µL loopful) to give $1\text{-}2 \times 10^4$ CFU/plate. Reduce the inoculum ten-fold, if necessary, to obtain isolated colonies.[1]

Selective Media and Tubed Media

Dilute the cell suspension 1:10 in normal saline or purified water. Streak each plate with 0.01 mL (10 µL loopful) of the suspension to provide $1\text{-}2 \times 10^5$ CFU/plate. Reduce the inoculum ten-fold, if necessary, to avoid overwhelming some selective media.[1]

Special Applications

Media used for special applications should be qualified accordingly. For example, prior to using Fluid Thioglycollate Medium and/or Soybean-Casein Digest Medium (Tryptic Soy Broth and **Trypticase** Soy Broth) for sterility testing, the media should be tested for growth promotion according to the specifications outlined in the *United States Pharmacopeia*[2] or a comparable reference standard.

Results

For general-purpose (non-selective) media, sufficient, characteristic growth and typical colony morphology should be obtained with all test strains. For selective media, growth of designated organisms is inhibited and adequate growth of desired organisms is obtained. Color and hemolytic reaction criteria must be met.

References

1. Clinical and Laboratory Standards Institute. 2004. Approved standard M22-A3. Quality control for commercially prepared microbiological culture media, 3rd ed. CLSI, Wayne, Pa.
2. The United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. The United States Pharmacopeial Convention, Inc., Rockville, Md.

Typical Analyses

"Typical" chemical compositions have been determined on the peptones and hydrolysates used in microbiological culture media. The typical analysis is used to select products for research or production needs when specific nutritional characteristics are required. The specifications for the typical analysis include:

- Nitrogen content
- Physical characteristics
- Inorganics
- Amino acids

For typical peptone and hydrolysate analyses, refer to the table in the Reference Guide section of this manual. To obtain Certificates of Analysis on specific lots of peptones and hydrolysates, visit http://regdocs.bd.com.

Glossary and Methods

Nitrogen Content

Total nitrogen (TN) was measured using a modified Kjeldhal digestion method. Not all organic nitrogen is nutritive. Percent (%) nitrogen × 6.25 ≅ % proteins, peptides or amino acids present.

The **amino nitrogen** (AN) value shows the extent of protein hydrolysis by measuring the increase in free amino groups. This is a nutritionally meaningful value.

The **AN/TN** ratio gives an estimate of the degree of protein hydrolysis.

Physical Characteristics

Ash: The higher the ash content, the lower the clarity of the prepared ingredient. Ash values refer to the non-combustible portion of the sample and roughly correspond to the mineral content of the sample. The ash content includes sodium chloride, sulfate, phosphates, silicates and metal oxides. Acid-insoluble ash is typically from silicates found in animal fodder. Ash values were measured after a minimum of 4 hours heating at 600°C.

Moisture (Loss on Drying): Lower moisture levels (<5%) are preferred. Higher moisture levels in dehydrated ingredients may reduce stability. In the presence of high moisture and high ambient temperatures, chemical interactions will cause darkening of the product and falling pH. These characteristics indicate product deterioration. Loss on Drying determinations were based on the method described in the *United States Pharmacopoeia*[1] (with some modifications to the procedure).

NaCl: The sodium chloride (NaCl) content may reflect significant pH adjustments during processing; e.g., acid hydrolysates (see Ash). Sodium Chloride was determined by silver nitrate/potassium thiocyanate titration method.

pH: pH was measured in a 2% solution after autoclaving and equilibrating to room temperature. Hydrolysates vary in their pH resistance according to their inherent buffering (phosphate) capacity.

Inorganics

Elemental analysis (calcium, magnesium, potassium, sodium) was determined by ICP (Inductively Coupled Plasma) using a Thermo Jarrell Ash instrument.

Phosphate, chloride and sulfate percentages were determined by ion chromatography.

Amino Acids

Free Amino Acids are defined as amino acids that are not part of a protein or peptide chain. The amino acids were measured using the Waters AccQ•Tag™* Method. The AccQ•Tag™ Method is based on the derivatizing reagent 6-aminoquinolyl-N-hydroxysuccinimide-activated heterocyclic carbamate.

Total Amino Acids were measured by the same method as the Free Amino Acids after an acid hydrolysis at 110°C for 20 hours. Cysteine and tryptophan are destroyed during the hydrolysis. The cysteine and tryptophan values are not reported for Total Amino Acids.

Reference

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Section II

General Technical Information

Dehydrated Culture Media

I. Introduction

BD supplies greater than 400 different **Difco**™ and **BBL**™ brand media formulations in dehydrated form for the convenience of the laboratorian and bioprocessing specialist. Certain formulations are designated by a "II" in the medium name; these formulations are optimized to provide more consistent, superior test results than earlier versions.

The individual ingredients and the final dehydrated product are carefully quality-controlled to assure consistent, high quality performance and obviate the need for media to be prepared in the laboratory from raw materials. A number of factors should be considered in the use of dehydrated media to ensure the quality of the finished product. These factors are discussed below.

Dehydrated culture media may be offered as 100 g and 500 g bottles, 2 kg, 5 lb, 25 lb and 10 kg containers.

II. Formula(e)

Generally, media ingredients are listed according to published "classical" formulations. However, since biological raw materials may vary slightly, formulations are listed as "approximate" and may be adjusted and/or supplemented to meet performance criteria.

Peptones in the formulations presented in this manual may be expressed by their traditional names or by their generic equivalents. The *United States Pharmacopeia* lists culture media peptones by generic names and includes specifications for peptone (meat peptone), pancreatic digest of casein, peptic digest of animal tissue and papaic digest of soybean meal. The peptones in **BBL** brand media have been converted to their generic equivalents. In general, the peptones in **Difco** brand media have retained their traditional names, which also appear in the published formulations in many textbooks and in "standard" references containing methods for the microbiological examination of foods, dairy products and waters and wastewaters.

Traditionally, the amount of an infusion listed for an infusion-containing medium has been expressed as the weight of the source material (e.g., Beef Heart, Infusion from 250 g) used in the infusion extraction process. These values have been converted to their dry weight equivalents in this manual, which is consistent with their use in dehydrated culture media.

III. Warnings and Precautions

Dehydrated culture media are For Laboratory Use.

Sodium azide is a selective agent in certain culture media. Media containing sodium azide are very toxic by inhalation, in contact with skin and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, since it must be assumed that all specimens/samples collected might contain infectious microorganisms. After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding. Directions for use should be read and followed carefully.

To minimize the risk in microbiology laboratories of working with infectious microorganisms and specimens and samples suspected to contain them, the United States Department of Health and Human Services has published guidelines for handling these agents and materials.[1] The guidelines describe four biosafety levels, some of which are mentioned in this manual in association with specific microorganisms:

- **Biosafety Level 1** is applicable when work is done with defined and characterized strains of viable organisms not known to consistently cause disease in healthy adult humans.
- **Biosafety Level 2** practices are applicable to laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are associated with human disease; activities can be performed on the open bench provided the potential for producing splashes or aerosols is low.
- **Biosafety Level 3** practices are applicable to laboratories working with agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. All laboratory manipulations should be performed in a biological safety cabinet or other enclosed equipment to protect personnel and the environment from exposure to potentially infectious aerosols.
- **Biosafety Level 4** practices are applicable for work with highly dangerous agents which may be transmitted via the aerosol route, for which there is no available vaccine or therapy and for which specialized equipment and facilities are required.

Consult the reference for specific recommendations on the practices, equipment and facilities of the four biosafety levels.[1]

IV. Storage Instructions

The majority of formulations should be stored within the range of 2-25°C in a dry place at a distance from sources of heat and sunlight; for a few, storage at 2-8°C is required. Unless otherwise noted, media described in this manual should be stored at 2-25°C.

Dehydrated culture media are hygroscopic. When bottles of dehydrated media have been opened for initial use, they should be tightly closed as soon as possible to protect them from hydration.

Provision should be made for rotating the stock of dehydrated media to ensure product freshness, obviating the use of aged materials, and discarding of media which is outdated.

V. Expiration Date

The expiration date applies to the products in their intact containers when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

VI. Product Deterioration

Verify that the physical characteristics of the powder are typical. Hydration can lead to caking and/or microbial contamination which render the culture medium unusable.

VII. Specimen/Sample Collection and Transport

The success of a microbiological isolation procedure does not depend solely on the quality of the culture media utilized. Proper specimen/sample collection and transport are crucial steps in the isolation process. A variety of transport systems and holding media have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Clinical Specimens

For clinical specimens, **BBL™ CultureSwab™** and **Port-A-Cul™** collection and transport products are available. For transport and growth of the pathogenic *Neisseria*, Transgrow, Gono-Pak and JEMBEC™* systems containing Modified Thayer-Martin (MTM II), Martin-Lewis or **GC-Lect™** Agars are recommended. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

The clinical laboratory must be furnished with sufficient patient information to enable the microbiologist to select the most suitable media and appropriate techniques. For detailed information, appropriate references should be consulted.

** JEMBEC is a trademark of Miles Scientific.*

Industrial Samples

Sterile containers should be used to collect samples. For environmental monitoring, samples can be collected using BD Sterile Pack Swabs.

Samples must represent the mass of the material being examined. Samples may require special handling, including refrigeration, to prevent the direct contamination of the sample by microorganisms and the subsequent growth of such contaminants during sampling, transportation and storage before examination. For detailed information, appropriate references should be consulted.

VIII. Materials Provided

Dehydrated Culture Medium
Supplements and Enrichments (if applicable)

IX. Materials Required But Not Provided

Glassware
Measuring scale
pH meter
Autoclave
Purified water, pH 5.5-7.5
Water bath
Incubator
Sterile Petri dishes or tubes
Ancillary culture media, reagents and laboratory equipment as required.

X. Procedures

Rehydration

The procedure employed for dissolving dehydrated culture media very often determines the clarity and performance of the finished product. Homogeneity of the solution and minimal heat exposure are important considerations.

Prior to use, examine the dehydrated material. Caked or discolored material should not be used for the preparation of culture media batches.

Add the precise amount of powdered material to approximately one-half of the volume of purified water. After thorough mixing, add the remainder of the water with care being taken to wash down the sides of the container (preferably an Erlenmeyer flask that is at least 2-3× the volume of medium). Dissolution is enhanced by allowing agar preparations to stand for 5 minutes with occasional agitation prior to boiling. Formulae that do not contain agar, gelatin or cystine will dissolve without heating, but heat is required to dissolve others so that they can be dispensed and sterilized. When heating is required, heat should be applied gently and the preparation agitated as required to prevent scorching. However, care should be taken to avoid media eruptions that may occur when agitating a flask of medium which is at or very near the boiling point. Boil as briefly as possible to obtain solution; 1 minute is usually sufficient. Exposure for longer periods can darken the medium and severely reduce its growth promotion properties. In no case should powdered medium be added to water and immediately placed into an autoclave since layering and separation of ingredients, precipitate formation and darkening are likely to occur with diminution of performance.

Sterilization

Follow label directions for length and temperature of sterilization. The recommended sterilization times assume a volume of one liter (1000 mL) or less. For larger volumes, the sterilization time should be extended but the temperature should not

be raised. When larger volumes are used, validation studies should be performed to determine the optimum sterilization cycle for each unique container size/volume combination. Autoclave media containing carbohydrates at a temperature not exceeding 116-118°C to avoid caramelization of the carbohydrate. It is important that physical parameters of the sterilizer and the efficacy of kill be monitored frequently through the use of calibration instrumentation and biological indicators.

Do not autoclave media that should not be heat-sterilized. There are numerous formulations available that can merely be dissolved and used directly. The performance of such media is seriously impaired by subjecting them to heat.

Adding Enrichments and Supplements

Enrichments and supplements tend to be heat sensitive. Cool the medium to 45-55°C in a water bath prior to adding enrichments or supplements. Ensure adequate mixing of the basal medium with enrichments or supplements by swirling to mix thoroughly.

Sterile broths may be cooled to room temperature before adding enrichment.

pH

Commercial dehydrated culture media are designed to fall within the specified pH range *after* steam sterilization.

For filter sterilization, adjust the pH, if necessary, prior to filtering.

Measure pH at room temperature (25°C). Avoid excessive pH adjustments.

Dispensing and Storage of Prepared Media

After sterilization, media prepared in the laboratory should be removed from the autoclave as soon as the pressure has fallen to zero. Hastening the opening of the autoclave before zero pressure is reached can result in the ejection of media from the sterilization vessels with considerable loss of contents.

For preparing plates, cool the medium to 50-55°C prior to dispensing to reduce water evaporation. Ensure gentle mixing during dispensing and dispense quickly. If using an automatic plate dispenser, dispense general-purpose media before dispensing selective media. Invert filled and cooled (solidified) Petri dish bottoms over their off-set lids and allow to sit for 1-2 hours to obtain a dry surface. It is advisable to use freshly poured plated media on the day of preparation. Alternatively, plates should be placed in the refrigerator as soon as they have solidified (agar side up) and several representative plates incubated at 35 ± 2°C as a sterility check. If storage of plates is for more than several days, it is recommended that they be wrapped in plastic or otherwise protected to prevent moisture loss. Most media, and especially those containing dyes or indicators, should be protected from light during storage.

When they have cooled, tighten the caps of media that are contained in screw-capped tubes or bottles; store all tubes and bottles under appropriate conditions, usually at ambient room temperature. The shelf life of some media, such as Lowenstein-Jensen Medium, may be prolonged by refrigeration.

Prepared media that have been refrigerated should be removed from refrigeration and equilibrated to room temperature prior to inoculation to allow water of condensation to evaporate or dissipate and to avoid temperature shock to the inoculum.

XI. User Quality Control

For media prepared in-house, each lot of every medium must be tested. When qualifying a new lot of culture medium, always test the new lot of medium in parallel with an approved lot of medium. Quality control organisms must be maintained appropriately and inoculum preparation should be performed according to published guidelines (refer to the monograph "Quality Control Organisms"). Maintain appropriate records and report deficiencies to the manufacturer.

Comments on BD User Quality Control

In the product descriptions, the User Quality Control section contains procedures for identity (Identity Specifications) and performance (Cultural Response). Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco** and **BBL** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

For Identity Specifications, the expected appearance of the powder, and of the solution following the addition of the powder to purified water and boiling, when appropriate, are indicated. The prepared (finished) medium appearance and final pH, both determined at 25°C, are specified.

For Cultural Response, test organisms, inocula and results are provided. Except for those media which are tested with fresh cultures (undiluted agar or broth cultures), the number of colony forming units (CFU) per plate is provided. Under Recovery, growth on **Difco** and **BBL** plated media may be reported as None, Poor (growth in quadrant one), Fair (growth in quadrants one and two) and Good (growth in quadrants three and four). Similar terms may be used for tubed media. The cultures listed are the minimum that should be used for performance testing.

For media referenced in Chapters <61> and <62> of the recently harmonized *United States Pharmacopeia*, the "User Quality Control" section contains the information required to verify that these media were tested according to the *USP, EP* and *JP* — **for both dehydrated culture media and prepared culture media products.** In other words, the media listed under "Availability" and identified with a staff mark (†), have been tested and meet *USP, EP* and *JP* performance specifications where applicable.

XII. Limitations of the Procedure

Detergent residues can compromise media. After washing glassware, check for alkali or acid residue with a few drops of bromthymol blue pH indicator (yellow is acidic; blue is alkaline).

Quantities of media in excess of one liter may require an extended autoclave time to achieve sterilization. Longer sterilization cycles can cause nutrient concentration changes and generation of inhibitory substances.

Since the nutritional requirements of organisms vary, a single medium is rarely adequate for detecting all organisms of potential significance in a clinical specimen or industrial sample. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species are present in large numbers in the specimen/sample. Cultures of specimens/samples grown on selective media should, therefore, be compared with specimens/samples cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens and other significant organisms.

XIII. Reference

1. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

PROBLEM	A	B	C	D	E	F	G	H	OTHER CAUSES
Abnormal color of medium	•	•	•						
Incorrect pH	•	•	•	•	•	•	•		Storage at high temperature Hydrolysis of ingredients pH determined at wrong temperature
Atypical precipitate	•	•	•	•	•	•			
Incomplete solubility					•				Inadequate heating Inadequate convection in a too small flask
Darkening or carmelization	•			•	•	•			
Toxicity		•	•						Burning or scorching
Trace substances (Vitamins)		•							Airborne or environmental sources of vitamins
Loss of gelation property				•	•	•		•	Hydrolysis of agar due to pH shift Not boiling medium
Loss of nutritive value or selective or differential properties	•		•	•	•	•	•	•	Burning or scorching Presence of strong electrolytes, sugar solutions, detergents, antiseptics, metallic poisons, protein materials or other substances that may inhibit the inoculum
Contamination	•	•	•						Improper sterilization Poor technique in adding enrichments and pouring plates Not boiling agar containing medium

Key

A Deteriorated Dehydrated Medium
B Improperly Washed Glassware
C Impure Water
D Incorrect Weighing
E Incomplete Mixing
F Overheating
G Repeated Remelting
H Dilution by a Too Large Inoculum

Prepared Plated Media

I. Introduction

Since 1960, BD has been a leading manufacturer of prepared plated media. **BBL**™ prepared plated media consist of **BBL**™, and in some cases **Difco**™, dehydrated culture media prepared and poured into disposable Petri dishes ready for immediate use. Each lot of **BBL** prepared plated media is manufactured from pretested components, and the final product is quarantined during quality control testing.

BBL plated media primarily are supplied in easy-open packages of twenty (20) and cartons of one hundred 100 × 15 mm-style **Stacker**™ dishes, designed to interlock to minimize the hazard of sliding stacks. Some media are supplied in packages of 10 dishes.

Prepared **Stacker**™ **I Plate**™ dishes and bi-plate dishes (with marked halves) contain either the same medium in each side of the dish divider or two different media for selective isolation or differentiation of microorganisms.

The 150 × 15 mm-style dish is offered in packages of 8 or boxes of 24 dishes containing Mueller Hinton Agar, with and without blood, and other media for use in the standardized Bauer-Kirby method of antimicrobial susceptibility testing.

NOTE: The catalog numbers for prepared plates listed under "Availability" for each of the media descriptions are 100 × 15 mm-style unless another size is indicated.

Prepared Plated Media for Environmental Monitoring

BD **RODAC**™ (Replicate Organism Detection and Counting) and Contact plates are 60 mm-style plates used for surface sampling for microbial contamination. The base of the **RODAC**™ plate has inward-sloping walls and a convex bottom designed to keep the agar bed in place during transit and use. The **RODAC**™ SL (Secure Lid) has three lugs on the base, providing a tight fit between lid and base to reduce accidental contamination.

In addition, BD **Hycheck**™ hygiene contact slides are used to assess the microbiological contamination of surfaces or fluids. Contained in a tightly sealed tube with screw cap, the double-sided design features a hinged paddle that bends for easy sampling; one paddle produces selective and nonselective results or surface samples can be obtained from two separate sites.

BD also offers four lines of prepared sterile pack plated media for critical environments. Because the entire double-bagged product is subjected to a sterilizing dose of gamma irradiation, the contents inside the outer bag are sterile (refer to the monograph "Media Sterilization"). This allows the inner bag to be aseptically removed and brought into an environmentally-controlled area without introducing contaminants. A third sterile rolled-up inner bag is included and may be employed as a transport container from the critical environment. The four options offered are:

- Sterile Pack **RODAC**™ plates for monitoring the microbial load on impervious surfaces.
- Sterile Pack Settling Plates for air sampling; these plates fit a variety of viable particle air sampler instruments.
- Sterile Pack **Finger Dab**™ Plates for sampling gloved hands.
- Sterile Isolator Pack Plates are similar to the above except that the multi-wrap design has been validated to protect the medium from the vaporized hydrogen peroxide used in the isolator decontamination cycle.

II. Formulae

Formulae for **BBL**™ brand prepared plated media are included in product inserts or the ***BBL***™ *Quality Control and Product Information Manual for Plated and Tubed Media*.

III. Warnings and Precautions

Prepared plated media are For *in vitro* Diagnostic Use or For Laboratory Use as labeled.

Directions for use should be read and followed carefully.

If excessive moisture is observed, invert the bottom over an offset lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, since it must be assumed that all specimens/samples collected might contain infectious microorganisms. After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

To minimize the risk in microbiology laboratories of working with infectious microorganisms and specimens and samples suspected to contain them, the United States Department of Health and Human Services has published guidelines for handling these agents and materials.[1] The guidelines describe four biosafety levels, some of which are mentioned in this manual in association with specific microorganisms:

- **Biosafety Level 1** is applicable when work is done with defined and characterized strains of viable organisms not known to consistently cause disease in healthy adult humans.
- **Biosafety Level 2** practices are applicable to laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are associated with human disease; activities can be performed on the open bench provided the potential for producing splashes or aerosols is low.

- **Biosafety Level 3** practices are applicable to laboratories working with agents with a potential for respiratory transmission and which may cause serious and potential lethal infection. All laboratory manipulations should be performed in a biological safety cabinet or other enclosed equipment to protect personnel and the environment from exposure to potentially infectious aerosols.
- **Biosafety Level 4** practices are applicable for work with highly dangerous agents which may be transmitted via the aerosol route, for which there is no available vaccine or therapy and for which specialized equipment and facilities are required.

Consult the reference for specific recommendations on the practices, equipment and facilities of the four biosafety levels.[1]

IV. Storage Instructions

On receipt, store plates at 2-8°C. Freezing and overheating must be avoided. Allow the medium to warm to room temperature before inoculation. Media containing dyes should be protected from light.

V. Expiration Date

Prepared plates stored in their original sleeve wrapping at 2-8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times, including up to 6 weeks for mycology media and up to 8 weeks for mycobacteriology media.

VI. Product Deterioration

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VII. Specimen/Sample Collection and Transport

The success of a microbiological isolation procedure does not depend solely on the quality of the culture media utilized. Proper specimen/sample collection and transport are crucial steps in the isolation process. A variety of transport systems and holding media have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Clinical Specimens

For clinical specimens, **BBL™ CultureSwab™** and **Port-A-Cul™** collection and transport products are available. For transport and growth of the pathogenic *Neisseria*, Transgrow, Gono-Pak and JEMBEC™* systems containing Modified Thayer-Martin (MTM II), Martin-Lewis or **GC-Lect™** Agars are recommended. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

The clinical laboratory must be furnished with sufficient patient information to enable the microbiologist to select the most suitable media and appropriate techniques. For detailed information, appropriate references should be consulted.

** JEMBEC is a trademark of Miles Scientific.*

Industrial Samples

Sterile containers should be used to collect samples. For environmental monitoring, samples can be collected using BD Sterile Pack Swabs.

Samples must represent the mass of the material being examined. Samples may require special handling, including refrigeration, to prevent the direct contamination of the sample by microorganisms and the subsequent growth of such contaminants during sampling, transportation and storage before examination. For detailed information, appropriate references should be consulted.

VIII. Materials Provided

Prepared plated medium

IX. Materials Required But Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

X. Procedures

Prepared plated media are ready to use with no additional preparation required. Allow plates to warm to room temperature prior to inoculation.

For anaerobic media, plates should be reduced immediately prior to inoculation by placing under anaerobic conditions for 18-24 hours. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak™** or **GasPak™** EZ anaerobic systems.

XI. User Quality Control

Quality control procedures for **BBL™** brand prepared plated media are included in product inserts or the ***BBL™ Quality Control and Product Information Manual for Plated and Tubed Media***.

If a culture medium being subjected to quality-control testing is a formulation to which the Clinical and Laboratory Standards Institute (CLSI) standard, *Quality Control for Commercially Prepared Microbiological Culture Media*,[2] applies, American Type Culture Collection (ATCC™) control strains specified by the document are utilized; additional ATCC and other organism strains may be also employed. If no standard exists for the particular medium, the organisms used represent strains from our stock culture collection. Except for CLSI-specified strains, cultures employed in testing procedures may be added or changed from time to time as strains are found that provide a greater challenge. Clinical isolates are included periodically for various formulations so as to check performance with "wild" strains.

To determine pH, bring the medium to room temperature (25°C), transfer the medium from the plate to a beaker,

macerate and immerse the electrode. Alternatively, a pH electrode designed for flat surfaces may be used.

XII. Limitations of the Procedure

Some diagnostic tests may be performed with the primary plate (e.g., **BBL**™ **CHROMagar**™ media). However, a pure culture is recommended for the majority of biochemical tests and other identification procedures. Consult appropriate references for further information.

Since the nutritional requirements of organisms vary, a single medium is rarely adequate for detecting all organisms of potential significance in a clinical specimen or industrial sample. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species are present in large numbers in the specimen/sample. Cultures of specimens/samples grown on selective media should, therefore, be compared with specimens/samples cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens and other significant organisms.

XIII. References

1. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
2. Clinical and Laboratory Standards Institute. 2004. Approved standard: M22-A3, Quality control for commercially prepared microbiological culture media, 3rd ed. CLSI, Wayne, Pa.

Prepared Tubed, Bottled and Mycoflask™ Media

I. Introduction

Since 1952, BD has been a leading manufacturer of prepared tubed and bottled media. **BBL**™ prepared tubed and bottled media consist of **BBL**™ and, in some cases **Difco**™, dehydrated culture media prepared and poured into a variety of sizes ready for immediate use. Some formulations are also supplied in **Mycoflask** bottles which provide a greater surface area for inoculation. Each lot of **BBL** and **Difco** prepared tubed, bottled and **Mycoflask** media is manufactured from pretested components and the final product is quarantined during quality control testing.

Prepared Tubed Media

BBL prepared tubed media are provided in several sizes of glassware depending on the application of the individual culture medium. Catalog listings indicate the type of tube in each instance. Dimensions of each of the tubes are shown below.

A tube size	20 × 148 mm with cap
C tube size	20 × 112 mm with cap
D tube size	16.5 × 128 mm with cap
K tube size	16 × 102 mm with cap

Tubes are borosilicate glass with black screw caps securely fitted to prevent loss of moisture. A special resilient rubber liner is affixed inside each cap with a unique adhesive that resists displacement even during autoclaving. Larger-sized tubes permit easy inoculation or streaking of media. Prepared slanted media are supplied in tubes with optimal dimensions that provide a large surface for inoculation. This is especially desirable in examining specimens for the presence of *Mycobacterium tuberculosis*.

The "A" tubes containing solid media (e.g., **Trypticase**™ Soy Agar), except slants, contain a quantity sufficient to pour one plate; this is approximately 20 mL. "A" tube slants for cultivation on the agar surface contain approximately 9 mL of medium slanted at an angle to yield a slope of approximately 10 cm in length. "A" tubes containing liquid culture medium such as Fluid Thioglycollate Medium, unless otherwise noted, are filled to contain approximately 20 mL.

The "C" tube is used primarily for cultivation of fungi and mycobacteria.

The "D" tube is utilized for certain general-purpose and special-purpose media. These are filled usually with approximately 10 mL of medium. When filled with differential agars, such as TSI Agar, approximately 7 mL are used to produce the characteristic long butt with short slant needed for these special-purpose media.

The "K" tube provides the convenience of a wide mouth with vertical shelf space-saving. Unless otherwise noted, slants contain approximately 4 mL, slants with butts approximately 6 mL and broths approximately 5-8 mL.

Media are available in packages of 10 and cartons of 100 tubes to meet the volume needs of various users.

Prepared Bottled Media

BBL and **Difco** prepared bottled media are available in a variety of sizes and closures. Color-coded caps are used for easy product identification and transparent labels allow clear view of media and cultures. In addition, for sterility testing, we now offer Sterile Pack bottles in two formulations: Tryptic Soy Broth and Fluid Thioglycollate Medium. Sterile Pack Bottles, which are validated sterile at a Sterility Assurance Level (SAL) of 10^{-6}, are terminally sterilized inside of autoclavable double-bags, resulting in a bottle exterior that is free from environmental contaminants and particulate matter. Bottled media products conform to the *United States Pharmacopeia*, *European Pharmacopoeia* and *Japanese Pharmacopoeia* requirements, as applicable.

BBL and **Difco** prepared bottled media are supplied in packages of 10 in a variety of fill volumes.

Mycoflask Bottles

Mycoflask media are disposable, ready-to-use **BBL** culture media prepared in the special **Mycoflask** bottle. A limited

variety of media for isolation and cultivation of tubercle bacilli and fungi is offered. The **Mycoflask** bottle's special features include:

- A deep offset well to contain a deep culture bed
- Surface area of 5.4 cm^2 for cultivation
- Horizontal incubation to flood agar surface with inoculum
- Flat-sided bottle which keeps bottle stable, reduces chances of breakage
- Tightly fitted screw caps which prevent the loss of moisture even when incubation is extended to 8 weeks or more

Mycoflask media are packaged in specially designed trays for ease in handling and incubation; these Unit Boxes contain 10 bottles. The Shelf-Pack contains 10 Unit Boxes (10 × 10 bottles).

II. Formulae

Formulae for **BBL** and **Difco** brands of prepared tubed, bottled and **Mycoflask** media are included in product inserts or the *BBL™ Quality Control and Product Information Manual for Plated and Tubed Media* or on product carton labels (bottled media).

III. Warnings and Precautions

These media are For *in vitro* Diagnostic Use or For Laboratory Use as labeled.

Directions for use should be read and followed carefully.

Care should be exercised in opening tubes and bottles with tight caps to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, since it must be assumed that all specimens/samples collected might contain infectious microorganisms. The use of a biohazard cabinet is recommended when working with pure cultures and specimens/samples suspected to contain fungi or mycobacteria. After use, prepared tubes, bottles or **Mycoflask** bottles, specimen/sample containers and other contaminated materials must be sterilized before discarding.

To minimize the risk in microbiology laboratories of working with infectious microorganisms and specimens and samples suspected to contain them, the United States Department of Health and Human Services has published guidelines for handling these agents and materials.[1] The guidelines describe four biosafety levels, some of which are mentioned in this manual in association with specific microorganisms:

- **Biosafety Level 1** is applicable when work is done with defined and characterized strains of viable organisms not known to consistently cause disease in healthy adult humans.
- **Biosafety Level 2** practices are applicable to laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are associated with human disease; activities can be performed on the open bench provided the potential for producing splashes or aerosols is low.
- **Biosafety Level 3** practices are applicable to laboratories working with agents with a potential for respiratory transmission and which may cause serious and potential lethal infection. All laboratory manipulations should be performed in a biological safety cabinet or other enclosed equipment to protect personnel and the environment from exposure to potentially infectious aerosols.
- **Biosafety Level 4** practices are applicable for work with highly dangerous agents which may be transmitted via the aerosol route, for which there is no available vaccine or therapy and for which specialized equipment and facilities are required.

Consult the reference for specific recommendations on the practices, equipment and facilities of the four biosafety levels.[1]

IV. Storage Instructions

On receipt, media should be stored according to label instructions. Freezing and overheating must be avoided. Allow the medium to warm to room temperature before inoculation.

V. Expiration Date

The expiration date applies to intact tubes and bottles stored as directed. Do not open until ready to use. Tubed and bottled media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times, including up to 6 weeks for mycology media and up to 8 weeks for mycobacteriology media.

VI. Product Deterioration

Do not use tubes or bottles if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VII. Specimen/Sample Collection and Transport

The success of a microbiological isolation procedure does not depend solely on the quality of the culture media utilized. Proper specimen/sample collection and transport are crucial steps in the isolation process. A variety of transport systems and holding media have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Clinical Specimens

For clinical specimens, **BBL™ CultureSwab™** and **Port-A-Cul™** collection and transport products are available. For transport and growth of the pathogenic *Neisseria*, Transgrow, Gono-Pak and JEMBEC™* systems containing Modified Thayer-Martin (MTM II), Martin-Lewis or **GC-Lect™** Agars are recommended. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

The clinical laboratory must be furnished with sufficient patient information to enable the microbiologist to select the most suitable media and appropriate techniques. For detailed information, appropriate references should be consulted.

** JEMBEC is a trademark of Miles Scientific.*

Industrial Samples

Sterile containers should be used to collect samples. For environmental monitoring, samples can be collected using BD Sterile Pack Swabs.

Samples must represent the mass of the material being examined. Samples may require special handling, including refrigeration, to prevent the direct contamination of the sample by microorganisms and the subsequent growth of such contaminants during sampling, transportation and storage before examination. For detailed information, appropriate references should be consulted.

VIII. Materials Provided

Prepared tubed or bottled medium.

IX. Materials Required But Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

X. Procedures

In some tubes, the agar may become distorted during shipment (e.g., semisolid formulations used for motility studies). Additionally, media (including all thioglycollate-containing media) may become oxidized within the tube or bottle during shipment. These can be restored to their proper condition by bringing to 100°C in a boiling water bath and resolidifying in the appropriate position; screw caps should be slightly loosened prior to boiling. The boiling also serves to reduce media intended for the cultivation of anaerobic organisms; caps should be tightened during cooling to room temperature. Consult product label for specific instructions.

Tubed media in deeps (pour tubes) must be boiled, cooled to 45-50°C, poured into sterile Petri dishes and allowed to harden for a minimum of 30 minutes prior to use.

NOTE: Use of a microwave oven to melt tubed and bottled media is *not* recommended.

Cultures requiring prolonged incubation, for example, mycobacteria and fungi, should be incubated with caps tightly closed to prevent dehydration and consequent inhibition of growth.

The *United States Pharmacopeia* requires that Fluid Thioglycollate Medium and Soybean-Casein Digest Medium (Tryptic Soy Broth and **Trypticase**™ Soy Broth) be incubated under aerobic conditions. Aerobic conditions can be maintained during incubation by insertion of a venting needle that is left in place during incubation or by incubating the tubes or bottles with the caps slightly loosened.

XI. User Quality Control

Quality control procedures for **BBL**™ brand prepared tubed and **Mycoflask** media are included in product inserts or the ***BBL***™ *Quality Control and Product Information Manual for Plated and Tubed Media*.

If a culture medium being subjected to quality-control testing is a formulation to which the Clinical and Laboratory Standards Institute (CLSI) standard, *Quality Control for Commercially Prepared Microbiological Culture Media*,[2] applies, American Type Culture Collection (ATCC™) control strains specified by the document are utilized; additional ATCC and other organism strains may be also employed. If no standard exists for the particular medium, the organisms used represent strains from our stock culture collection. Except for CLSI-specified strains, cultures employed in testing procedures may be added or changed from time to time as strains are found that provide a greater challenge. Clinical isolates are included periodically for various formulations so as to check performance with "wild" strains.

An uninoculated tube of medium always should be incubated with the inoculated tubes for purposes of comparison (e.g., color changes, turbidity) following the incubation period. This procedure should be adopted both for quality control and test specimen evaluations.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed and **Mycoflask** media. The tip of the electrode should be placed below the surface of broth media, and positioned in the central portion of the agar mass in semisolid or solid media. Warm all media to room temperature (25°C) prior to measuring pH.

XII. Limitations of the Procedure

Some diagnostic tests may be performed with the primary culture. However, a pure culture is recommended for biochemical tests and other identification procedures. Consult appropriate references for further information.

Since the nutritional requirements of organisms vary, a single medium is rarely adequate for detecting all organisms of potential significance in a clinical specimen or industrial sample. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species are present in large numbers in the specimen/sample. Cultures of specimens/samples grown on selective media should, therefore, be compared with specimens/samples cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens and other significant organisms.

Culture media sometime contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon

Gram staining include staining reagents, immersion oil, glass slides and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

XIII. References

1. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
2. Clinical and Laboratory Standards Institute. 2004. Approved standard: M22-A3, Quality control for commercially prepared microbiological culture media, 3rd ed. CLSI, Wayne, Pa.

Section III

Culture Media and Ingredients



A-1 Medium

Intended Use

A-1 Medium is used for detecting fecal coliforms in water.

Summary and Explanation

Since the early 1900s enumeration of coliform organisms, specifically *Escherichia coli*, has been used to determine water purity. Elevated-temperature, most-probable-number (MPN) methods are routinely used for the analysis of water and food samples for the presence of fecal coliforms. One limiting factor in using *E. coli* is the length of time required for complete identification.[1] A-1 Medium was formulated to hasten the recovery of *E. coli* and reduce the incidence of false positive cultures.

In 1972 Andrews and Presnell developed A-1 Medium. A-1 Medium recovers *E. coli* from estuarine water in 24 hours instead of 72 hours, and in greater numbers without the preenrichment step.[2] Using a 3-hour preincubation step for the enumeration of coliforms in chlorinated wastewater gave results that were statistically comparable to those obtained in the two-step MPN technique.[3]

A-1 Medium can be used in a single-step procedure for the detection of fecal coliforms in source water, seawater, treated wastewater and foods. Prior enrichment in a presumptive medium is not required.[4] A-1 Medium conforms to standard methods for the isolation of fecal coliforms in water and foods.[4,5]

Principles of the Procedure

Peptone provides the nitrogen, vitamins, minerals and amino acids in A-1 Medium. Lactose is the carbon source and, in combination with salicin, provides energy for organism growth. Sodium chloride maintains the osmotic balance of the medium. Triton™* X-100 is a surfactant.

**Triton is a trademark of Rohm and Haas Company.*

Formula

Difco™ A-1 Medium

Approximate Formula* Per Liter		
Tryptone	20.0	g
Lactose	5.0	g
Sodium Chloride	5.0	g
Salicin	0.5	g
Triton X-100	1.0	mL

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 31.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 10 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: For 10 mL water samples, prepare double-strength medium to ensure ingredient concentrations are not reduced below those of the standard medium.[4]

User Quality Control

Identity Specifications

Difco™ A-1 Medium

Dehydrated Appearance:	Light beige, lumpy.
Solution:	3.15% solution, soluble in purified water upon boiling. Solution is light amber, opalescent immediately after autoclaving. Upon cooling clear, may have a flocculent precipitate.
Prepared Appearance:	Light amber, clear, may have a flocculent precipitate.
Reaction of 3.15% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

Difco™ A-1 Medium

Prepare the medium per label directions. Prepare tubes by placing fermentation vials and 10 mL amounts of medium into tubes. Inoculate and incubate at 35 ± 2°C for 3 hours. Transfer tubes to a 44.5°C water bath for 21 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS
Bacillus subtilis	6633	10^2	None	–
Enterobacter aerogenes	13048	10^2	Poor to good*	–
Enterococcus faecalis	19433	10^2	None to poor	–
Escherichia coli	25922	10^2	Good	+
Escherichia coli	13762	10^2	Good	+

**May or may not produce gas.*

Uninoculated Tube

Escherichia coli ATCC™ 25922 with fermentation vial

Procedure

1. Inoculate tubes of A-1 Medium as directed in standard methods.[4,5]
2. Incubate at 35 ± 0.5°C for 3 hours.
3. Transfer tubes to a water bath at 44.5 ± 0.2°C and incubate for an additional 21 ± 2 hours.
4. Maintain water level in bath above level of liquid in inoculated tubes.

Expected Results[4,5]

Gas production in the inverted vial, or dissolved gas that forms fine bubbles when slightly agitated, is a positive reaction indicating the presence of fecal coliforms. Calculate fecal coliform densities using MPN tables from standard methods.

Limitations of the Procedure

1. Fecal coliform counts are usually greater than *E. coli* counts.[5]
2. Interpretation of test procedure using A-1 Medium requires understanding of the microflora of the specimen.[5]

References

1. Andrews, Diggs and Wilson. 1975. Appl. Microbiol. *29*:130.
2. Andrews and Presnell. 1972. Appl. Microbiol. *23*:521.
3. Standridge and Delfino. 1981. Appl. Environ. Microbiol. *42*:918.
4. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ A-1 Medium

COMPF EPA SMWW

Cat. No. 218231 Dehydrated – 500 g

A7 Agar, Modified

Intended Use

A7 Agar, Modified is used for the cultivation, identification and differentiation of *Ureaplasma urealyticum* and other members of the genus *Ureaplasma* from other members of the *Mycoplasmatales*.

Summary and Explanation

As knowledge and interest increased regarding the group of organisms now known as *Mycoplasma* but originally referred to as PPLOs (pleuropneumonia-like organisms), a number of different culture media were developed to support the growth of these unique organisms. One of these, designated A7 Agar, was formulated by Shepard and Lunceford.[1,2] The performance of the medium has been improved by the addition of growth factors.

Uninoculated Plate

The medium is particularly useful for detection and identification of *U. urealyticum* in primary cultures of urine and urethral exudates. The medium, by incorporation of a direct test for urease in colonies of *Ureaplasma*, provides for a simple and rapid differentiation of the genus from other *Mycoplasmatales*.

Principles of the Procedure

Mycoplasmas require a highly nutritious growth medium, which is provided by the peptones, yeast extract, growth factors, horse serum, dextrose, salts and constituents of **BBL™ IsoVitaleX™** Enrichment in A7 Agar, Modified. The medium contains added urea and a sensitive indicator of ammonia (manganese sulfate) to provide differentiation by incorporation of the biochemical principle of the direct spot test for urease in colonies of *Ureaplasma*.[1] The presence of urease enzyme hydrolyzes urea with the production of ammonia and resultant alkalinity, which precipitates the metallic oxide product (MnO_2) within and on the surface of *Ureaplasma* colonies.[2] Penicillin G is included in the formulation to provide for inhibition of gram-positive endogenous bacteria.[3,4]

Procedure

Using an extract from swabs, or the specimen itself, streak the surface of the medium. Incubate plates in a moist anaerobic atmosphere supplemented with CO_2 (**BD GasPak™** EZ container or equivalent system) at 35-37° C. Incubate plates for 48 hours or longer. Examine plates for colonies of *U. urealyticum* with the culture plate inverted on the microscope stage, under low power (100×).[2]

Expected Results

Isolated colonies should give results consistent with those of the quality control stains. Colonies of *U. urealyticum* are small (usually 16-18 mm), dark, golden brown or deep brown with a light background color of the medium. Species of *Ureaplasma*

are the only members of the *Mycoplasmatales* known to produce urease, upon which the specific color reaction depends.[1,2]

References

1. Shepard and Lunceford. 1976. J. Clin. Microbiol. 3:613.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Shepard. 1983. In Razin and Tulley (ed.), Methods in mycoplasmology, vol. 1. Academic Press, Inc., New York, N.Y.
4. Waites and Taylor-Robinson. 1999. In Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ A7 Agar, Modified

Cat. No. 292211 Prepared Plates (60 × 15 mm-style) – Pkg. of 10*

**Store at 2-8°C.*

AC Broth

Intended Use

AC Broth is used for cultivating a wide variety of microorganisms and for the sterility testing of turbid or viscous solutions and other materials not containing mercurial preservatives.

Summary and Explanation

AC Broth possesses growth-promoting properties for a wide variety of microorganisms. Christensen[1] and Malin and Finn[2] reported that AC Medium does not exhibit the toxicity shown by media containing sodium thioglycollate. Several early studies reported on the wide variety of organisms able to grow on AC Medium.[3-5] AC Broth is suitable for use in the detection of obligately aerobic contaminants in biologicals and other products. AC Broth is also useful in the isolation and cultivation of many common pathogenic and saprophytic aerobes.[6] The medium can be used to test the sterility of biologicals and solutions that do not contain mercurial preservatives. Fluid Thioglycollate Medium should be employed for the sterility testing of solutions containing mercurial preservatives.

User Quality Control

Identity Specifications

Difco™ AC Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.4% solution, soluble in purified water. Solution is medium to dark amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 3.4% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ AC Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Corynebacterium diphtheriae biotype mitis	8024	10^2-10^3	Good
Streptococcus mitis	9895	10^2-10^3	Good
Streptococcus pneumoniae	6305	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

Principles of the Procedure

Peptone, beef extract and malt extract provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon are provided by yeast extract. Dextrose is a carbon energy source. Ascorbic acid is added to clarify the solution.

Formula

Difco™ AC Broth

Approximate Formula* Per Liter		
Proteose Peptone No. 3	20.0	g
Beef Extract	3.0	g
Yeast Extract	3.0	g
Malt Extract	3.0	g
Dextrose	5.0	g
Ascorbic Acid	0.2	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 34 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Store prepared medium at 15-30°C.
4. After prolonged storage, reheat in flowing steam or a boiling water bath for a few minutes to drive off dissolved gases. Cool without agitation.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

When reheating prepared media to drive off dissolved gases, do not overheat because this may result in decreased growth.

References

1. Christensen. 1944. Paper read at New York Meeting. American Public Health Association.
2. Malin and Finn. 1951. J. Bacteriol. *62*:349.
3. Reed and Orr. 1943. J. Bacteriol. *45*:309.
4. Schneiter, Dunn and Caminita. 1945. Public Health Rep. *60*:789.
5. Kolb and Schneiter. 1950. J. Bacteriol. *59*:401.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ AC Broth

Cat. No. 231710 Dehydrated – 500 g

AK Agar #2 (Sporulating Agar)

Intended Use

AK Agar #2 (Sporulating Agar) is a culture medium for the preparation of spore suspensions for use in procedures for the detection of antibiotic residues in milk and dairy products.

Summary and Explanation

AK Agar #2 was devised by Arret and Kirshbaum for specific use in the production of spores of *Bacillus subtilis* ATCC™ 6633 for use in the Penicillin Milk Test procedure.[1] This medium was formerly specified in the spore preparation phase of the American Public Health Association disc assay procedure for the detection of sulfa drugs and antibiotics in milk.[2]

Principles of the Procedure

The peptones and beef extract are sources of nitrogen, sulfur, amino acids and essential trace ingredients. Yeast extract is a rich source of B vitamins. Dextrose is an energy source for bacterial replication. Manganous sulfate plays an important role in the sporulation process.

Formula

BBL™ AK Agar #2

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	6.0	g
Pancreatic Digest of Casein	4.0	g
Yeast Extract	3.0	g
Beef Extract	1.5	g
Dextrose	1.0	g
Agar	15.0	g
Manganous Sulfate	0.3	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 30.8 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. *For preparation of spore suspensions for use in the FDA procedure for the Penicillin Milk Test.*[1]
   Transfer cultures of *Bacillus subtilis* ATCC 6633 monthly to fresh Seed Agar slants. Wash the growth from a fresh slant culture with sterile physiological saline onto the surface of a Roux bottle containing 300 mL of AK Agar #2. Incubate the bottles for 5 days at 35 ± 2°C and wash off the resulting growth into 50 mL of sterile physiological saline. Centrifuge the suspension and decant and discard the supernatant fluid. Resuspend the sediment in sterile saline and heat shock the suspension at 70°C for 30 minutes. The resultant spore suspension can be stored for several months. Consult the reference for the test procedure utilizing this *B. subtilis* spore suspension.[1]
2. *For preparation of spore suspension for use in the APHA procedure for detection of sulfa drugs and antibiotics in milk.*[2]
   Transfer cells of *Bacillus megaterium* ATCC 9855 by streaking the entire surface of sterile AK Agar #2 contained in a prescription (180 mL capacity) or Roux bottle. Incubate inoculated bottles at 35 ± 2°C for 48 hours. After incubation, wash the spores and vegetative cells from the agar surface with buffered MS (microbiologically suitable) water. Sediment the spores and cells by centrifugation at 5,000 × *g* for 15 minutes at 3°C. Store the spore suspension in buffered MS water under refrigeration. Consult the reference for the test procedure utilizing this *B. megaterium* spore suspension.[2]

User Quality Control

Identity Specifications

BBL™ AK Agar #2

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.08% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to moderately hazy.
Reaction of 3.08% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

BBL™ AK Agar #2

Prepare the medium per label directions. Inoculate plates and incubate at 35 ± 2°C for 18-24 hours. Reincubate plates at 35 ± 2°C and prepare slides after 2 days (and again after 5 days for *B. subtilis* only).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	SPORE PRODUCTION
Bacillus megaterium	9855	10^2-10^3	Good	+
Bacillus subtilis	6633	10^2-10^3	Good	+

Expected Results

Suspensions containing large numbers of bacterial spores are obtained with the use of AK Agar #2.

Availability

BBL™ AK Agar #2 (Sporulating Agar)

Cat. No. 210912 Dehydrated – 500 g

References

1. Arret and Kirshbaum. 1959. J. Milk Food Technol. 22:329.
2. Richardson (ed.). 1985. Standard methods for the examination of dairy products, 15th ed. American Public Health Association. Washington, D.C.

APT Agar • APT Broth

Intended Use

APT Agar is used for cultivating heterofermentative lactobacilli and other organisms requiring high thiamine content. It is also used for maintaining stock cultures of *Weissella (Lactobacillus) viridescens* ATCC™ 12706 used in the assay of thiamine.

APT Broth is used for culturing *Weissella viridescens* ATCC 12706 used in the assay of thiamine. It is also used for cultivating heterofermentative lactobacilli and other organisms requiring high thiamine content.

Summary and Explanation

Evans and Niven[1] investigated cultivating the heterofermentative lactobacilli that cause the faded or greenish discoloration of cured meat products, while Deibel, Evans and Niven[2] investigated thiamine requiring bacteria, specifically *Lactobacillus viridescens*. Their formulations led to the development of APT Agar and APT Broth.

Historically, the lactic acid bacteria, a group of acid-producing bacteria, included the genera *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Lactobacillus*; currently, taxonomists include a number of additional genera (e.g., *Weissella*).[3] These organisms are widespread in nature and are associated with bacterial spoilage of foods such as dairy, meat and vegetable products.[3] One use of APT Agar and APT Broth is for cultivating these heterofermentative lactic acid bacteria from food products.[3]

APT Agar and APT Broth are also used in the microbiological assay of thiamine. In the assay, APT Agar is the maintenance medium that preserves the viability and sensitivity of *Weissella viridescens* ATCC 12706. APT Broth is used for growing *Weissella viridescens* ATCC 12706 and preparing the inoculum.

User Quality Control

Identity Specifications

Difco™ APT Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.12%, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 6.12% Solution at 25°C:	pH 6.7 ± 0.2

Difco™ APT Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.62%, soluble in purified water with slight heating. Solution is opalescent when hot. After cooling, is light to medium amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent without significant precipitate.
Reaction of 4.62% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ APT Agar or APT Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus fermentum	9338	10^2-10^3	Good
Weissella viridescens	12706	10^2-10^3	Good

Principles of the Procedure

APT Agar and APT Broth contain peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate. The manganese chloride, magnesium sulfate and ferrous sulfate provide ions used in replication by lactobacilli. Polysorbate 80 is a source of fatty acids required by lactobacilli. Agar is the solidifying agent in APT Agar.

Formulae

Difco™ APT Agar

Approximate Formula* Per Liter

Yeast Extract	7.5	g
Pancreatic Digest of Casein	12.5	g
Dextrose	10.0	g
Sodium Citrate	5.0	g
Thiamine Hydrochloride	1.0	mg
Sodium Chloride	5.0	g
Dipotassium Phosphate	5.0	g
Manganese Chloride	0.14	g
Magnesium Sulfate	0.8	g
Ferrous Sulfate	0.04	g
Polysorbate 80	0.2	g
Agar	15.0	g

Difco™ APT Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ APT Agar

1. Suspend 61.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ APT Broth

1. Suspend 46.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For maintaining stock cultures of *Weissella viridescens* ATCC 12706 prepare a stab inoculation. Prepare stock cultures in triplicate at monthly intervals. One of the transfers is saved for the preparation of stock cultures. The others are used to prepare inoculum in APT Broth for assay as needed. Following incubation at 35-37°C for 24-48 hours, store stock cultures at 2-8°C.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Evans and Niven. 1951. J. Bacteriol. *62*:599.
2. Deibel, Evans and Niven. 1957. J. Bacteriol. *74*:818.
3. Hall, Ledenbach and Flowers. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ APT Agar

COMPF USDA

Cat. No. 265430 Dehydrated – 500 g

Difco™ APT Broth

Cat. No. 265510 Dehydrated – 500 g

Acetamide Agar

Intended Use

Acetamide Agar is used in the differentiation of nonfermentative gram-negative bacteria, particularly *Pseudomonas aeruginosa*.

Summary and Explanation

Assimilation studies by Gilardi and others using basal mineral media showed that acetamide was utilized by a wide variety of nonfermenting organisms.[1,2] However, few organisms are reported to deaminate acetamide.[3,4] A variety of media formulations have been developed to determine the ability of various nonfermenting gram-negative organisms to deaminate acetamide for purposes of identification.[5-8] The formulation of this medium is the one recommended in *Standard Methods for the Examination of Water and Wastewater*.[9]

Principles of the Procedure

The ability to deaminate acetamide (acylamidase activity) has been found to be most actively accomplished by *P. aeruginosa*, *Comamonas acidovorans*, *Achromobacter xylosoxidans* subsp. *xylosoxidans* (*Alcaligenes xylosoxidans*) and *Alcaligenes faecalis (odorans)*.[8] Deamination of acetamide produces ammonia which increases the pH of the medium causing a corresponding color change from yellow-orange to purplish-red.

Procedure

Inoculate the Acetamide Agar slant with a loopful of culture emulsified in BBL™ Trypticase™ Soy Broth. Incubate inoculated slant at 35 ± 2°C and observe daily for 4 days and again at 7 days before discarding as negative.

Expected Results

Deamination of the acetamide is indicated by a pronounced purplish-red color of the medium.

Complete identification requires determination of the Gram reaction, cellular morphology, biochemical reactions, etc. Appropriate references may be consulted for further information.[10, 11]

Limitations of the Procedure

Some strains deaminate acetamide slowly and may require as long as 7 days to yield a positive test result.

Only about 37% of apyocyanogenic strains of *P. aeruginosa* will produce a positive reaction. Therefore, this test should not be relied upon as a sole criterion for identification.[11]

References

1. Gilardi. 1974. Antonie van Leewenhoek. J. Microbiol. Serol. *39*:229.
2. Stainier, Palleroni and Doudoroff. 1966. J. Gen. Microbiol. *43*:159.
3. Pickett and Pedersen. 1970. Can. J. Microbiol. *16*:351.
4. Pickett and Pedersen. 1970. Can. J. Microbiol. *16*:401.
5. Hedberg. 1969. Appl. Microbiol. *17*:481.
6. Smith and Dayton. 1972. Appl. Microbiol. *24*:143.
7. Buhlmann, Vischer and Bruhin. 1961. J. Bacteriol. *82*:787.
8. Oberhofer and Rowen. 1974. Appl. Microbiol. *28*:720.
9. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
10. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
11. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Acetamide Agar

SMWW

Cat. No. 221828 Prepared Slants – Pkg. of 10*

**Store at 2-8°C.*

Acetate Differential Agar

Intended Use

Acetate Differential Agar is used for the differentiation of *Shigella* species from *Escherichia coli*.

Summary and Explanation

Organic acids have been used widely as an aid to the differentiation of *Enterobacteriaceae*, usually in formulae that contained organic nitrogen sources. Most bacteria, however, can use citrate and acetate in the presence of organic nitrogen.

The citrate media of Koser[1] and Simmons[2] were free of organic nitrogen and, therefore, were a true measure of citrate utilization. In a further extension of this approach, Trabulsi and Ewing developed Acetate Differential Agar, a chemically defined medium utilizing sodium acetate that enables the differentiation of *Shigella* spp. from *E. coli*, particularly anaerogenic, nonmotile biotypes.[3,4] Their basal medium was Simmons Citrate Agar in which sodium acetate was substituted for sodium citrate.

Principles of the Procedure

Acetate Differential Agar consists of a mixture of salts and sodium acetate, as a sole source of carbon, in a chemically defined medium devoid of organic nitrogen.

Typical cultures of *Shigella* are unable to utilize acetate and fail to grow; therefore, the medium remains unchanged. Most cultures of *E. coli* and closely related organisms grow well within 24-48 hours, but some strains grow more slowly and a few cannot use the acetate as a source of carbon. The blue color of the bromthymol blue is due to the production of alkaline products from the utilization of the sodium acetate.

Formula

Difco™ Acetate Differential Agar

Approximate Formula* Per Liter

Sodium Acetate	2.0	g
Magnesium Sulfate	0.1	g
Sodium Chloride	5.0	g
Monoammonium Phosphate	1.0	g
Dipotassium Phosphate	1.0	g
Bromthymol Blue	0.08	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 29.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense into tubes to allow a 10 mm butt and a 30 mm slant.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the agar slant surfaces with pure cultures of unknown organisms. Incubate all tubes for up to 7 days at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Bacteria capable of utilizing acetate as the sole carbon source will grow on the medium and produce an alkaline reaction (blue color). For a listing of organisms capable of utilizing acetate, consult appropriate texts.[4-6]

Limitations of the Procedure

Some strains of *E. coli* utilize acetate slowly or not at all and may give a false-negative reaction. Sodium acetate is utilized as a sole source of carbon by some biotypes of *S. flexneri* 4a.[4]

User Quality Control

Identity Specifications

Difco™ Acetate Differential Agar

Dehydrated Appearance:	Medium yellowish-tan to light green, free-flowing, homogeneous.
Solution:	2.92% solution, soluble in purified water upon boiling. Solution is emerald green, slightly opalescent.
Prepared Appearance:	Emerald green to green, slightly opalescent.
Reaction of 2.92% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Acetate Differential Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 2-7 days. Acetate utilization is indicated by a color change of the slant from green to blue.

ORGANISM	ATCC™	RECOVERY	ACETATE UTILIZATION
Escherichia coli	25922	Good	Positive (blue)
Shigella sonnei	25931	Poor to good	Negative (green)

Uninoculated Tube

Escherichia coli ATCC™ 25922

References

1. Koser. 1923. J. Bacteriol. *8*:493.
2. Simmons. 1926. J. Infect. Dis. *39*:209.
3. Trabulsi and Ewing. 1962. Public Health Lab. *20*:137.
4. Ewing. 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Acetate Differential Agar

BAM COMPF SMD

Cat. No. 274210 Dehydrated – 500 g

BBL™ Acetate Differential Agar

BAM COMPF SMD

Cat. No. 221375 Prepared Slants – Pkg. of 10

Acidicase™ Peptone

(See Casamino Acids)

Actinomyces Broth

Intended Use

Actinomyces Broth is used as a liquid medium or, with the addition of 7 or 20 g/L of agar, as a semisolid or solid medium, respectively, for the maintenance or cultivation of *Actinomyces* species.

Summary and Explanation

Actinomyces Broth is a basic medium modified from the Actinomyces Maintenance Medium of Pine and Watson.[1] It is recommended for use in the growth and maintenance of members of the genus *Actinomyces*.[2]

Principles of the Procedure

Actinomyces Broth contains meat infusion, peptone, yeast extract, soluble starch, L-cysteine and dextrose, which provide carbon, nitrogen, sulfur, vitamins and other growth factors required for the metabolism of *Actinomyces* spp. The salts provide essential minerals and electrolytes.

User Quality Control

Identity Specifications

BBL™ Actinomyces Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.7% solution, soluble in purified water. Solution is light to medium, yellow to tan, trace hazy to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, trace hazy to moderately hazy.
Reaction of 5.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

BBL™ Actinomyces Broth

Prepare the medium per label directions. Inoculate and incubate anaerobically at 35 ± 2°C for 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Actinomyces bovis	13683	<10^3	Growth
Actinomyces israelii	10049	<10^3	Growth

Formula

BBL™ Actinomyces Broth

Approximate Formula* Per Liter

Heart Muscle, Infusion from (solids)	2.0	g
Pancreatic Digest of Casein	17.0	g
Yeast Extract	10.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	13.0	g
Monopotassium Phosphate	2.0	g
Dextrose	5.0	g
Ammonium Sulfate	1.0	g
L-Cysteine HCl	1.0	g
Soluble Starch	1.0	g
Magnesium Sulfate	0.2	g
Calcium Chloride	0.01	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 57 g of the powder in 1 L of purified water. Add agar, 7 or 20 g/L, if a semisolid or solid medium is desired.
2. If agar is added, heat with frequent agitation just until solution occurs.
3. Dispense and autoclave at 121°C for 10 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate *Actinomyces* cultures into tubes containing broth, semisolid or solid media. The semisolid medium should be stab-inoculated and the slanted medium should be inoculated over its entire surface.

Incubate cultures at 35 ± 2°C in an anaerobic atmosphere (BD GasPak™ EZ anaerobic system, or alternative system for the cultivation of anaerobic microorganisms).

Expected Results

After growth is obtained, tubes containing broth may be frozen for long-term storage. Cultures grown in the semisolid medium can be refrigerated after growth has been obtained. Agar slant cultures are for use in a relatively short period of time.

References

1. Pine and Watson. 1959. J. Lab. Clin. Med. 54:107.
2. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994. U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Actinomyces Broth

Cat. No. 210920 Dehydrated – 500 g

Actinomycete Isolation Agar Glycerol

Intended Use

Actinomycete Isolation Agar is used with added glycerol for isolating and cultivating actinomycetes from soil and water.

Glycerol is used in preparing microbiological culture media.

Summary and Explanation

Although some genera are important to human medicine, most of the actinomycetes are part of the indigenous flora of soil, water and vegetation. Actinomycetes may impart a musty odor to water or a muddy flavor to fish.[1] Actinomycetes can cause massive growths which will form a thick foam in the activated sludge process, causing a disruption in wastewater treatment.[2,3] Actinomycetes are gram-positive, acid-fast cells, growing as filaments that may branch and may form irregularly shaped rods and cocci.

Olsen[4] formulated Actinomycete Isolation Agar for isolating and cultivating actinomycetes from soil and water. The formula is supplemented with glycerol, a highly purified fermentable alcohol used occasionally for differentiating certain bacteria and in media for isolating and culturing fastidious bacteria.

Principles of the Procedure

Actinomycete Isolation Agar contains sodium caseinate which is a source of nitrogen. Asparagine is an amino acid and a source of organic nitrogen. Sodium propionate is a substrate used in anaerobic fermentation. Dipotassium phosphate provides buffering capability to maintain pH balance. Magnesium sulfate and ferrous sulfate provide sources of sulfates and metallic ions. Agar is the solidifying agent. The added glycerol is a source of carbon.

User Quality Control

Identity Specifications

Difco™ Actinomycete Isolation Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.2% solution, soluble in purified water upon boiling with 0.5% Glycerol. Solution is light to medium amber, opalescent to opaque with precipitation.
Prepared Appearance:	Medium amber, opalescent.
Reaction of 2.2% Solution with 0.5% Glycerol at 25°C:	pH 8.1 ± 0.2

Cultural Response

Difco™ Actinomycete Isolation Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Streptomyces achromogenes	12767	10^2-10^3	Good
Streptomyces albus	3004	10^2-10^3	Good
Streptomyces lavendulae	8664	10^2-10^3	Good

Formulae

Difco™ Actinomycete Isolation Agar

Approximate Formula* Per Liter

Sodium Caseinate	2.0	g
Asparagine	0.1	g
Sodium Propionate	4.0	g
Dipotassium Phosphate	0.5	g
Magnesium Sulfate	0.1	g
Ferrous Sulfate	1.0	mg
Agar	15.0	g

Difco™ Glycerol

Glycerin

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 22 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 5 g of Glycerol.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate medium and incubate at 30°C for up to 72 hours.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
2. Lechevalier. 1975. Actinomycetes of sewage-treatment plants. Environ. Protection Technol. Ser., EPA-600/2-75-031, U. S. Environmental Protection Agency, Cincinnati, Ohio.
3. Lechevalier and Lechevalier. 1974. Int. J. Syst. Bacteriol. 24:278.
4. Olsen. 1960. Personal communication.

Availability

Difco™ Actinomycete Isolation Agar

Cat. No. 212168 Dehydrated – 500 g

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

Agars
Bacto™ Agar • Agar, Grade A • Agar, Granulated Agar, Technical • Agar, Noble • Agarose • Agar, Select

Intended Use

Bacto™ Agar is a solidifying agent in which extraneous matter, pigmented portions and salts have been reduced to a minimum. **Bacto** Agar is used in preparing microbiological culture media.

Agar, Grade A is a high-grade agar, specially processed for microbiological purposes. It is routinely used as a solidifying agent in microbiological media.

Agar, Granulated is a solidifying agent used in preparing microbiological culture media.

Agar, Technical is a solidifying agent used in preparing microbiological culture media. Although Agar, Technical has wider quality control parameters than other bacteriological agars, solubility, gelation temperature and solidity are carefully monitored to permit its use.

Agar, Noble is a solidifying agent that is essentially free of impurities. It is used in electrophoretic and nutritional procedures and in preparing microbiological culture media when increased purity is required.

Agarose is a complex galactose polysaccharide of near neutral charge. It is specially prepared and is intended mainly for use in gel electrophoresis.

Agar, Select is recommended for molecular genetics testing.

Summary and Explanation

Agar is a phycocolloid extracted from a group of red-purple marine algae (Class Rhodophyceae) including *Gelidium*, *Pterocladia* and *Gracilaria*. *Gelidium* is the preferred source for agars. Impurities, debris, minerals and pigment are reduced to specified levels during manufacture.

Agar was first suggested for microbiological purposes in 1881 by Fannie Hesse.[1,2] By the early 1900s, agar became the gelling agent of choice over gelatin because agar remains firm at growth temperatures for many pathogens. Agar is also generally resistant to a breakdown by bacterial enzymes. The use of agar in microbiological media significantly contributed to the advance of microbiology, paving the way for pure culture isolation and study.

Agar is a gel at room temperature, remaining firm at temperatures as high as 65°C.[3] Agar melts at approximately 85°C, a different temperature from that at which it solidifies, 32-40°C. This property is known as hysteresis. Agar is generally resistant to shear forces; however, different agars may have different gel strengths or degrees of stiffness.

Agar is typically used in a final concentration of 1-2% for solidifying culture media. Smaller quantities (0.05-0.5%) are used in media for motility studies (0.5% w/v) and for growth of anaerobes (0.1%) and microaerophiles.[3]

The use of small amounts of agar in media for sterility testing was recommended by Falk et al.[4] and has been incorporated into Fluid Thioglycollate Medium for sterility testing by standard procedures.[5]

Specifications for bacteriological grade agar include good clarity, controlled gelation temperature, controlled melting temperature, good diffusion characteristics, absence of toxic bacterial inhibitors and relative absence of metabolically useful minerals and compounds.

Principles of the Procedure

Bacto Agar is optimized for beneficial calcium and magnesium content. Detrimental ions such as iron and copper are reduced. **Bacto** Agar is recommended for clinical applications, auxotrophic studies, bacterial and yeast transformation studies and bacterial molecular genetics applications.[6,7]

Grade A Agar is a select grade of agar containing essential minerals for bacterial growth. When utilized as an ingredient, most media formulations demonstrate improved growth and test reactions.

Granulated Agar is qualified for culturing recombinant strains of *Escherichia coli* (HB101) and *Saccharomyces cerevisiae*. Agar, Granulated may be used for general bacteriological purposes where clarity is not a strict requirement.

Technical Agar is suitable for many bacteriological applications. This agar is not highly processed, has broader technical specifications than other agars and is not recommended for growth of fastidious organisms.

Noble Agar is extensively washed and bleached. This agar should be used for applications where extreme clarity and high purity are required. Noble Agar is suitable for immunodiffusion, some electrophoretic applications, and as a substrate for mammalian or plant tissue culture.

Agarose is the low sulfate, neutral gelling fraction of agar. During the fractionation of agar, the agarose-portion is separated from the highly charged polysaccharides (high sulfate, nongelling portion), purified and dried. Because of its method of preparation, Agarose is considerably purer than the special kinds of agar, with respect to ionic groups, rendering it more valuable for gel electrophoresis.[8] In addition to high chemical purity, Agarose must exhibit certain physical properties; e.g., high gel strength and high gel clarity.[8] The suggested concentration for use is 0.5-1.2%.

Select Agar is a key ingredient used in molecular genetics work for determining bacteriophage lambda titers.

User Quality Control

Identity Specifications

	BACTO™ AGAR	BBL™ AGAR, GRADE A	DIFCO™ AGAR, GRANULATED	DIFCO™ AGAR, TECHNICAL	DIFCO™ AGAR, NOBLE	BBL™ AGAROSE	BBL™ AGAR, SELECT
Dehydrated Appearance	Very light beige to light beige, free-flowing, homogeneous granules.	Light to medium, yellow-cream to cream-tan, homogenous, free of extraneous material.	Very light to light beige, free-flowing, homogeneous granules.	Very light to medium beige, free-flowing, homogeneous.	White to off-white, free-flowing, homogeneous fine granules.	White to light tan, homogeneous, free of extraneous material.	Light cream to tan, homogeneous, free of extraneous material.
Solution Concentration	1.5%	1.5%	1.5%	1.5%	1.5%	1.0%	1.5%
Solution Appearance*	Solution is very light amber, very slightly to slightly opalescent, may contain a small amount of black particles.	Solution is colorless to tan, slightly hazy (minute to small cream particles may be present).	Solution is very light amber, very slight to slightly opalescent.	Solution is very light to medium amber, slightly opalescent to opalescent.	Solution is colorless to pale yellow, clear to very slightly opalescent.	Solution is colorless.	Solution is pale to light yellow to tan, clear to moderately hazy (minute to fine cream particles may be present).
pH at 25°C	N/A	5.5-7.5	N/A	N/A	N/A	6.1-7.1	5.5-7.5
Loss on Drying (LOD)	16-20%	5-11%	≤ 20%	≤ 20%	≤ 20%	≤ 10%	5-10%
Ash[5]	≤ 6.5%	3.0-6.5%	≤ 6.5%	≤ 6.5%	≤ 2%	≤ 1.0%	2.0-6.5%
Calcium µg/g (ppm)	300-3,000 ppm	N/A	≤ 300-2,500 ppm	≤ 3,000 ppm	≤ 1,000 ppm	N/A	N/A
Magnesium µg/g (ppm)	50-1,000 ppm	N/A	≤ 50-1,000 ppm	≤ 1,300 ppm	≤ 400 ppm	N/A	N/A
Melting Point	83-89°C	80-90°C	83-89°C	≥ 85°C	≥ 85°C	N/A	80-90°C
Gelation Point	32-39°C	33-38°C	32-39°C	32-39°C	32-39°C	N/A	33-38°C
Agar Gel Electrophoresis	N/A	N/A	N/A	N/A	Satisfactory	Satisfactory	N/A

**Soluble in purified water upon boiling.*

Cultural Response

Prepare the agar formulation of Nutrient Broth or LB Broth, Miller by adding 1.5% agar. Inoculate with 10^2-10^3 CFU of the indicated test organisms and incubate at 35 ± 2°C for 18-24 hours (18-72 hours for LB Broth, Miller). Record recovery.

	BACTO™ AGAR	DIFCO™ AGAR, GRANULATED	DIFCO™ AGAR, TECHNICAL	DIFCO™ AGAR,* NOBLE
Nutrient Broth with:				
Escherichia coli ATCC™ 25922	Good		Good	Good
Staphylococcus aureus ATCC™ 25923	Good		Good	Good
LB Broth, Miller with:				
Escherichia coli ATCC™ 33694 (HB101)		Good		
Saccharomyces cerevisiae ATCC™ 9763		Good		

**To evaluate for growth in tissue culture, prepare TC Medium 199 with 10% fetal calf serum and 0.5% Noble Agar. Adjust pH to 7.4-8.0. Inoculate tissue culture flasks with Vero cells and observe for attachment and division.*

BBL™ Agar, Grade A

This product is tested for satisfactory performance as plain **Trypticase**™ Soy Agar. Spread plates are inoculated in duplicate with serial dilutions of *Neisseria meningitidis* (ATCC™ 13090), *Streptococcus pneumoniae* (ATCC™ 6305) and *Streptococcus pyogenes* (ATCC™ 49117) such that one dilution contains 30-300 CFU/mL. Plates are incubated at 35 ± 2°C for 1 day with 3-5% CO_2. A satisfactory result corresponds to colony counts that are within 1.2 logs of an acceptable control lot.

BBL™ Agar, Select

This product is tested as NZC Bottom Agar and NZC Top Agar and tested for satisfactory propagation of bacteriophage lambda Charon 30 utilizing *Escherichia coli* ATCC 33526 (K802). To prepare NZC agars, add, per liter of purified water: Casitone, 10 g; Casamino Acids, 1.0 g; Sodium Chloride, 5.0 g; Magnesium Chloride (anhydrous), 0.94 g; for NZC Bottom Agar, add 9.0 of Select Agar; for NZC Top Agar, add 6.0 g of Select Agar.

Bacto™ Agar

Procedure

See appropriate references for specific procedures using **Bacto**™ Agar, Grade A Agar, Granulated Agar, Technical Agar, Noble Agar, Agarose or Select Agar.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Hesse. 1894. Mitt. a.d. Kaiserl. Gesh. Berlin 2:182.
2. Hitchens and Leikind. 1939. J. Bacteriol. 37:485.
3. Selby and Selby. 1959. Agar. *In* Whister (ed.), Industrial gums. Academic Press Inc., New York, N.Y.
4. Falk, Bucca and Simmons. 1939. J. Bacteriol. 37:121.
5. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
6. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning, a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York, N.Y.
7. Schiestl and Geitz. 1989. Current Genetics *16*:339.
8. Guiseley and Renn. 1975. Agarose: purification, properties, and biomedical applications. Marine Colloids, Inc. Rockland, Maine.

Typical Analyses

	BACTO™ AGAR	BBL™ AGAR, GRADE A	DIFCO™ AGAR, GRANULATED	DIFCO™ AGAR, TECHNICAL	DIFCO™ AGAR, NOBLE	BBL™ AGAROSE	BBL™ AGAR, SELECT
Physical Characteristics							
Concentration (%)	1.5	1.5	1.5	1.5	1.5	1.0	1.5
Ash (%)	3.6	3.0-6.5	3.4	4.1	1.3	< 1.0	2.0-6.5
Clarity (NTU)*	4.3	< 10	5.3	26.2	3.7	< 10	N/A
Color (430 nm, adsorbance)	N/A	< 0.2	N/A	N/A	N/A	< 0.2	N/A
Loss on Drying (%)	17.3	< 10	12.2	18.2	16.0	< 10	N/A
pH	6.5	5.5-7.5	6.6	6.9	5.7	6.0-7.0	5.5-7.5
Gel Strength (g/cm^2)	600	600-800	560	613	700	800-1200	N/A
Gelation Point (°C)	35	35-39	35	36	35	35-39	33-38
Melting Point (°C)	88	80-90	88	88	87	80-90	80-90
Resistivity (ohms)	N/A	N/A	N/A	N/A	N/A	> 50,000	N/A
-m, (electrophoretic)**	N/A	N/A	N/A	N/A	≤ 0.55	< 0.25	N/A
Inorganics (%)							
Calcium	0.179	0.23	0.133	0.110	0.015	0.03	N/A
Chloride	0.021	N/A	< 0.005	0.172	< 0.050	N/A	N/A
Cobalt	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Copper	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Iron	0.002	< 0.0060	0.003	0.002	< 0.001	< 0.0050	N/A
Lead	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Magnesium	0.068	0.10	0.041	0.093	0.002	0.01	N/A
Manganese	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Nitrate	< 0.005	N/A	< 0.005	< 0.005	< 0.050	N/A	N/A
Phosphate	< 0.005	0.02	0.010	0.015	< 0.050	0.08	N/A
Potassium	0.121	0.03	0.079	0.124	0.022	0.015	N/A
Sodium	0.837	1.8	0.776	0.932	0.335	< 0.1	N/A
Sulfate	1.778	N/A	1.710	0.367	0.663	N/A	N/A
Sulfur	0.841	0.7	0.868	0.646	0.333	0.1	N/A
Tin	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Zinc	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Biological Testing (CFU/g)							
Spore Count	< 1,000	≤ 20	< 1,000	4,300	< 1,000	N/A	N/A
Standard Plate Count	< 1,000	N/A	< 1,000	2,725	< 1,000	N/A	N/A

**Nephelometric turbidity units (NTU).*
***Unit of relative electroendosmosis.*

Availability

Bacto™ Agar

Cat. No. 214050 Dehydrated – 100 g
214010 Dehydrated – 454 g
214030 Dehydrated – 2 kg
214040 Dehydrated – 10 kg

BBL™ Agar, Grade A

Cat. No. 212304 Dehydrated – 454 g

Difco™ Agar, Granulated

Cat. No. 214530 Dehydrated – 500 g
214510 Dehydrated – 2 kg
214520 Dehydrated – 10 kg

Difco™ Agar, Technical

Cat. No. 281230 Dehydrated – 500 g
281210 Dehydrated – 2 kg

Difco™ Agar, Noble

Cat. No. 214220 Dehydrated – 100 g
214230 Dehydrated – 500 g

BBL™ Agarose

Cat. No. 212272 Dehydrated – 500 g

BBL™ Agar, Select

Cat. No. 299340 Dehydrated – 500 g
299341 Dehydrated – 5 lb (2.3 kg)

Agar, 1.5%

Intended Use

Agar, 1.5% is used in the alternative agar-overlay method of inoculation for antimicrobial disc diffusion susceptibility testing. It may also be used in other microbiological procedures, such as colony counts, that require an agar preparation free from additives.

Summary and Explanation

The Bauer-Kirby method of antimicrobial susceptibility testing is well standardized and accurate.[1] However, the method for standardizing the inoculum introduces some subjective variability.

In 1970, Barry et al. introduced an agar-overlay procedure as a modification of the method described by Bauer et al.[2] They found that placing the discs onto a double pour plate, consisting of a base layer of Mueller Hinton Agar overlaid with a thin layer of inoculated 1.5 percent agar produced more clearly defined zone edges for more precise zone measurements than the Bauer-Kirby method of placing the discs directly onto inoculated Mueller Hinton Agar. The overlay procedure also facilitated standardization of susceptibility testing by providing a reproducible inoculum.

Principles of the Procedure

These tubes contain bacteriological grade agar and purified water. When liquefied, by heating to 100°C and cooling to 45 to 50°C, the agar solution is used as a suspending matrix for bacteria that may be added to an appropriate plated medium and allowed to solidify. This procedure provides a method of evenly distributing an inoculum onto a plated medium.

Procedure

Because this product can be used for a variety of purposes, the instructions will vary according to use. For details on preparing the agar overlay for the disc diffusion technique of determining antimicrobial susceptibility, consult appropriate references.[2,3]

Expected Results

Following incubation of the plates, zones of inhibition surrounding the discs may be measured and compared with published standards.[3,4]

Limitations of the Procedure

The agar overlay method is not applicable to tests with fastidious or slow-growing organisms.[4] The method is not described in more recent editions of CLSI (NCCLS) standard M2.

References

1. Bauer, Kirby, Sherris and Turck. 1966. Am. J. Clin. Pathol. 45:493.
2. Barry, Garcia and Thrupp. 1970. Am. J. Clin. Pathol. 53:149.
3. Barry and Thornsberry. 1985. In Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
4. National Committee for Clinical Laboratory Standards. 1990. Approved standard M2-A4, Performance standards for antimicrobial disk susceptibility tests, 4th ed. NCCLS, Villanova, Pa.

Availability

BBL™ Agar, 1.5%

Cat. No. 297395 Prepared Tubes, 9 mL (D Tubes) – Ctn. of 100

Alkaline Peptone Water

Intended Use

Alkaline Peptone Water is an enrichment medium used for the cultivation of *Vibrio* species from feces and other infected materials.

Summary and Explanation

Clinical materials containing small numbers of *Vibrio* should be inoculated into an enrichment medium prior to plating onto a selective medium, such as TCBS Agar. Alkaline Peptone Water is a suitable enrichment broth for this purpose.[1-3] The relatively high pH of the medium (approximately 8.4) provides a favorable environment for the growth of vibrios.

Alkaline Peptone Water is recommended as an enrichment medium when analyzing food samples for *Vibrio*.[4] *Standard Methods for the Examination of Water and Wastewater* recommends its use as an enrichment medium for the cultivation of *Aeromonas* from water samples.[5]

Principles of the Procedure

Enzymatic digest of casein provides amino acids and other complex nitrogenous substances necessary to support bacterial growth. Sodium chloride maintains osmotic equilibrium.

Procedure

Swab specimens may be inserted directly into the medium. Material not being cultured from a swab may be transferred directly to the medium using a sterile inoculating loop. For fecal specimens, aseptically transfer approximately 1 g of the sample to the medium and mix well. If the feces are received in a preservative, transfer 2 to 3 mL of the fecal specimen to the medium.

Incubate at 35°C for 6-12 hours. Subculture onto a selective medium, such as TCBS Agar. Incubate subcultured plate at 35° C for 24-48 hours.

Expected Results

Growth in tubes is indicated by turbidity compared to an uninoculated control. Subculture growth onto selective and nonselective media for isolation and identification.

Limitations of the Procedure

This prepared tube medium is intended to be used as an enrichment medium. A pure culture is recommended for biochemical tests and other identification procedures.

References

1. Gilligan, Janda, Karmali and Miller. 1992. Cumitech 12A, Laboratory diagnosis of bacterial diarrhea. Coord. ed., Nolte. American Society for Microbiology, Washington, D.C.
2. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Isenberg and Garcia (ed.). 2004 (updated, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
4. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.

Availability

BBL™ Alkaline Peptone Water

BAM SMWW

Cat. No. 297814 Prepared Tubes, 8 mL (D Tubes) – Pkg. of 10

Amies Transport Media

(See Transport Media)

Amino Acid Assay Media
Lysine Assay Medium • Cystine Assay Medium

Intended Use

Lysine Assay Medium is used for determining lysine concentration by the microbiological assay technique.

Cystine Assay Medium is used for determining L-cystine concentration by the microbiological assay technique.

Summary and Explanation

Amino acid assay media are prepared for use in the microbiological assay of amino acids. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the amino acid under test. They contain all the factors necessary for optimal growth of the test organism except the single essential amino acid to be determined.

Amino Acid Assay Media are prepared according to the formulations of Steel et al.[1] They are used in the microbiological assay of amino acids using *Pediococcus acidilactici* ATCC™ 8042 as the test organism.

Principles of the Procedure

Lysine Assay Medium and Cystine Assay Medium contain all the factors essential for the growth of *Pediococcus acidilactici* ATCC 8042, except the amino acid under assay. The addition of the amino acid in specified increasing concentrations gives a growth response by the test organism.

Formulae[1]

Difco™ Lysine Assay Medium or Cystine Assay Medium

All amino acid assay media contain the following formula.

Approximate Formula* Per Liter		
Dextrose	50.0	g
Sodium Acetate	40.0	g
Ammonium Chloride	6.0	g
Monopotassium Phosphate	1.2	g
Dipotassium Phosphate	1.2	g
Magnesium Sulfate	0.4	g
Ferrous Sulfate	20.0	mg
Manganese Sulfate	40.0	mg
Sodium Chloride	20.0	mg
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Xanthine	20.0	mg
Thiamine Hydrochloride	1.0	mg
Pyrodoxine Hydrochloride	2.0	mg
Pyridoxamine Hydrochloride	600.0	mg
Pyridoxal Hydrochloride	600.0	mg
Calcium Pantothenate	1.0	mg
Riboflavin	1.0	mg
Nicotinic Acid	2.0	mg
p-Aminobenzoic Acid	200.0	µg
Biotin	2.0	µg
Folic Acid	20.0	µg
Glycine	0.2	g
DL-Alanine	0.4	g
Asparagine	0.8	g
L-Aspartic Acid	0.2	g
L-Proline	0.2	g
DL-Serine	0.1	g
DL-Tryptophan	80.0	mg
L-Glutamic Acid	0.6	g
L-Histidine Hydrochloride	124.0	mg
DL-Phenylalanine	0.2	g
DL-Threonine	0.4	g
L-Tyrosine	0.2	g
DL-Valine	0.5	g
DL-Isoleucine	0.5	g
DL-Leucine	0.5	g
L-Arginine Hydrochloride	484.0	mg
DL-Methionine	0.2	g

User Quality Control

Identity Specifications

Difco™ Lysine Assay Medium or Cystine Assay Medium

Dehydrated Appearance:	White to off-white, homogeneous, may have a tendency to clump.
Solution:	5.25% (single strength) solution, soluble in purified water upon boiling. Solution is light to medium amber, clear, may have a slight precipitate.
Prepared Appearance:	Single strength—Light to medium amber, clear, may have a slight precipitate
Reaction of 5.25% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Lysine Assay Medium or Cystine Assay Medium

Prepare the medium per label directions. These media support the growth of *Pediococcus acidilactici* ATCC™ 8042 when prepared in single strength and supplemented with the appropriate amino acid. Lysine Assay Medium should produce a standard curve when tested with L-Lysine at 0.0 to 300 µg per 10 mL. Cystine Assay Medium should produce a standard curve when tested with L-Cystine at 0 to 50 µg per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-20 hours. Read the percent transmittance at 660 nm.

Preparation of inoculum dilution, amino acid stock and working solution.

ASSAY MEDIUM	TEST CULTURE	PREPARATION OF INOCULUM DILUTION (CELL SUSPENSION + STERILE 0.85% NACl)	PREPARATION OF AMINO ACID STOCK SOLUTION (AMINO ACID + PURIFIED H_2O)		STANDARD WORKING SOLUTION (STOCK SOLUTION + PURIFIED H_2O)	VOLUME OF STANDARD WORKING SOLUTION (ML/10 ML TUBE)	FINAL AMINO ACID CONCENTRATION µG/10 ML
Lysine Assay Medium	*Pediococcus acidilactici* ATCC™ 8042	1 mL + 19 mL	L-lysine	6 g + 1,000 mL	1 mL + 99 mL	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 30, 60, 90, 120, 150, 180, 240, 300
Cystine Assay Medium	*Pediococcus acidilactici* ATCC™ 8042	1 mL + 19 mL	L-cystine	1 g + 100 mL + 1 mL HCl heated, then cooled, add up to 1,000 mL	1 mL + 99 mL	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 5, 10, 15, 20, 25, 30, 40, 50

In addition to the ingredients listed on the previous page, the media contain per liter*:

Lysine Assay Medium
L-Cystine.....0.1 g

Cystine Assay Medium
L-Lysine Hydrochloride.....0.5 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 10.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense in 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

Procedure

Stock Culture and Inoculum

Stock cultures of *Pediococcus acidilactici* ATCC 8042 are prepared by stab inoculation into tubes of Lactobacilli Agar AOAC or Micro Assay Culture Agar. Incubate cultures at 35-37°C for 24 hours. Store stock cultures at 2-8°C. Make transfers at monthly intervals in triplicate.

The inoculum for assay is prepared by subculturing the test organism into 10 mL Lactobacilli Broth AOAC or Micro Inoculum Broth. Incubate at 35-37°C for 16-24 hours. After incubation, centrifuge the cells under aseptic conditions and decant the liquid supernatant. Wash the cells 3 times with 10 mL sterile 0.85% NaCl solution. After the third wash, resuspend the cells in 10 mL sterile 0.85% NaCl solution. Dilute the 10 mL cell suspension with the appropriate amount of sterile 0.85% NaCl solution. (See the table under User Quality Control, Cultural Response.) One drop of the diluted inoculum suspension is used to inoculate each of the assay tubes.

Amino Acid Solution

Prepare stock solutions of each amino acid as described in the table under User Quality Control. Prepare the stock solutions fresh daily.

Increasing amounts of the standard or the unknown and sufficient purified water to give a total volume of 10 mL per tube are added to the tubes containing 5 mL of the rehydrated medium. The appropriate volumes of the standards and their final concentrations are listed in the table.

Measure the growth response turbidimetrically or titrimetrically. Turbidimetric readings are made after incubation at 35-37°C for 16-20 hours. Titrimetric readings are made after incubation at 35-37°C for 72 hours.

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation that influence the standard curve readings cannot always be duplicated.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of amino acid at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of amino acid in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

Reference

1. Steel, Sauberlich, Reynolds and Baumann. 1949. J. Biol. Chem. *177*:533.

Availability

Difco™ Lysine Assay Medium
Cat. No. 242210 Dehydrated – 100 g*

Difco™ Cystine Assay Medium
Cat. No. 246710 Dehydrated – 100 g*

**Store at 2-8°C.*

Anaerobe Broth MIC

(See Wilkins-Chalgren Agar)

Anaerobe CNA Agar with 5% Sheep Blood

Intended Use

Anaerobe CNA Agar with 5% Sheep Blood is used in a qualitative procedure for the selective isolation of anaerobic streptococci.

Summary and Explanation

Anaerobe CNA Agar with 5% Sheep Blood is an enriched medium based on a modified formulation for Columbia CNA Agar developed by Ellner et al.[1] It consists of Columbia CNA Agar modified to support the growth of anaerobic gram-positive cocci. Colistin and nalidixic acid (CNA) are incorporated to inhibit gram-negative enteric bacilli.

Principles of the Procedure

Anaerobe CNA Agar consists of Columbia Agar supplemented with L-cysteine and dithiothreitol as reducing agents and hemin and vitamin K_1 to supply nutrients necessary for the cultivation of anaerobes.[2]

The addition of antimicrobial agents facilitates the recovery of anaerobic microorganisms from specimens containing mixed flora. The incorporation of colistin and nalidixic acid enables the selective recovery of anaerobic cocci by inhibiting gram-negative enteric bacilli. The colistin disrupts the cell membranes of gram-negative organisms, whereas the nalidixic acid blocks DNA replication in susceptible gram-negative bacteria.[3]

The addition of defibrinated sheep blood supplies additional nutrients.

Procedure

The medium should be reduced immediately prior to inoculation by placing under anaerobic conditions for 6-24 hours.[4]

Incubate plates immediately after inoculation, with plates in an inverted position (agar side up), under anaerobic conditions at 35°C, or place the medium in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates is accumulated (no longer than 3 hours).[5] An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak™** EZ anaerobic systems or an alternative anaerobic system.

Incubate for at least 48 hours and, if no growth occurs, continue incubation for up to 7 days.

Expected Results

In order to determine the relationship to oxygen of each colony type, follow established procedures.[4,6] Those colony types that prove to be obligate anaerobes can be further studied using appropriate identification methods.[7]

References

1. Ellner, Granato and May. 1973. Appl. Microbiol. *26*:904.
2. Gibbons and MacDonald. 1960. J. Bacteriol. *80*:164.
3. Estevez. 1984. Lab. Med. *15*:258.
4. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. Martin. 1971. Appl. Microbiol. *22*:1168.
6. Mangels. 1994. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1., supp. 1. American Society for Microbiology, Washington, D.C.
7. Rodloff, Hillier and Moncla. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Anaerobe CNA Agar with 5% Sheep Blood

Cat. No. 297165 Prepared Plates – Pkg. of 20*

BBL™ Anaerobe CNA Agar with 5% Sheep Blood// Anaerobe Laked Sheep Blood KV Agar

Cat. No. 297041 Prepared **I Plate**™ Dishes – Pkg. of 20*

**Store at 2-8°C.*

Anaerobe Laked Sheep Blood KV Agar

Intended Use

Anaerobe Laked Sheep Blood KV Agar is used in a qualitative procedure for isolation of gram-negative anaerobic bacilli.

Summary and Explanation

Anaerobe Laked Sheep Blood KV Agar consists of an enriched Columbia Agar Base with kanamycin and vancomycin as selective agents for gram-negative obligate anaerobes. Laked sheep blood stimulates pigmentation of the pigmenting *Porphyromonas-Prevotella* species.

Principles of the Procedure

Anaerobe Laked Sheep Blood KV Agar consists of Columbia Agar supplemented with L-cysteine and dithiothreitol as reducing agents and hemin and vitamin K_1 to supply nutrients necessary for the cultivation of anaerobes.[1]

The addition of antimicrobial agents facilitates the recovery of anaerobic microorganisms from specimens containing mixed flora. The kanamycin and vancomycin enable the selective isolation of gram-negative anaerobic bacilli by inhibiting facultatively and obligately anaerobic gram-positive bacteria.[2]

Laking the blood improves pigmentation of the pigmenting *Porphyromonas-Prevotella* species.[3] Fluorescence is visible when the colonies are exposed to long-wave UV light.

Procedure

The medium should be reduced immediately prior to inoculation by placing under anaerobic conditions for 6-24 hours.[4]

Incubate plates immediately after inoculation, with plates in an inverted position (agar side up), under anaerobic conditions at 35°C, or place the medium in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates is accumulated (no longer than 3 hours).[5] An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak**™ EZ anaerobic systems or an alternative anaerobic system.

Incubate for at least 48 hours and, if no growth occurs, continue incubation for up to 7 days.

Expected Results

Examine colonies using a dissecting microscope and with a long-wave UV lamp to detect fluorescence. Colonies of the pigmenting *Porphyromonas-Prevotella* species should fluoresce orange to brick-red under long-wave UV light. Fluorescence is visible before pigmentation.

In order to determine the relationship to oxygen of each colony type, follow established procedures.[4,6] Those colony types that prove to be obligate anaerobes can be further studied using appropriate identification methods.

Limitation of the Procedure

The concentration of vancomycin (7.5 mg/mL) may be inhibitory to asaccharolytic *Porphyromonas* species.[7]

References

1. Gibbons and MacDonald. 1960. J. Bacteriol. *80*:164.
2. Finegold, Miller and Posnick. 1965. Ernahrungsforschung *10*:517.
3. Finegold and Citron. 1980. *In* Lennette, Balows, Hausler and Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
4. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. Martin. 1971. Appl. Microbiol. 22:1168.
6. Mangels. 1994. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1, suppl. 1. American Society for Microbiology, Washington, D.C.
7. Jousimies-Somer, Summanen and Finegold. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Anaerobe Laked Sheep Blood KV Agar// Anaerobe CNA Agar with 5% Sheep Blood

Cat. No. 297041 Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ Anaerobe Laked Sheep Blood KV Agar// CDC Anaerobe 5% Sheep Blood Agar with PEA

Cat. No. 299611 Prepared **I Plate**™ Dishes – Pkg. of 20*

**Store at 2-8°C.*

Anaerobe Neomycin 5% Sheep Blood Agar

Intended Use

Anaerobe Neomycin 5% Sheep Blood Agar is a selective medium used in qualitative procedures for the isolation and cultivation of fastidious and slow-growing obligately anaerobic bacteria from a variety of clinical and nonclinical specimens.

Summary and Explanation

Anaerobe Neomycin 5% Sheep Blood Agar consists of CDC Anaerobe 5% Sheep Blood Agar and the selective agent, neomycin. The CDC Anaerobe 5% Sheep Blood Agar is an enriched, nonselective medium suitable for the isolation and cultivation of a wide variety of obligately anaerobic gram-negative microorganisms.[1,2] The use of an antimicrobial agent improves the recovery of obligately anaerobic bacteria from specimens containing mixed flora.

Principles of the Procedure

CDC Anaerobe Blood Agar consists of **BBL™ Trypticase™** Soy Agar with additional agar, yeast extract, cystine, hemin and vitamin K_1. Defibrinated sheep blood is added to supply nutrients and for the determination of hemolytic reactions and pigmentation.

Neomycin inhibits the growth of most staphylococci and *Enterobacteriaceae*.

Procedure

The medium should be reduced immediately prior to inoculation by placing under anaerobic conditions for 6-24 hours.[3]

Incubate plates immediately after inoculation, with plates in an inverted position (agar side up), under anaerobic conditions at 35°C, or place the medium in a holding jar flushed with oxygen-free gas(es) until sufficient plates are accumulated (no longer than 3 hours).[4] An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak™** EZ anaerobic systems or an alternative system.

Incubate for at least 48 hours, and, if no growth occurs, continue incubation for up to 7 days. An indicator should be used to detect anaerobiosis.

Expected Results

After at least 48 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

In order to determine the relationship to oxygen of each colony type present on anaerobic solid media, follow established procedures.[3,5] The colony types that prove to contain obligate anaerobes can be further studied using appropriate identification methods.

References

1. Dowell, Lombard, Thompson and Armfield. 1977. Media for isolation, characterization and identification of obligately anaerobic bacteria. CDC laboratory manual. Center for Disease Control, Atlanta, Ga.
2. Dowell and Hawkins. 1987. Laboratory methods in anaerobic bacteriology, CDC laboratory manual. HHS Publication No. (CDC) 87-8272. Centers for Disease Control, Atlanta, Ga.
3. Allen, Siders and Marler. 1985. *In* Lenette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
4. Martin. 1971. Appl. Microbiol. 22:1168.
5. Mangels. 1994. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1, suppl. 1. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Anaerobe Neomycin 5% Sheep Blood Agar

Cat. No. 297790 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Anaerobic Agar

Intended Use

Anaerobic Agar is used for cultivating anaerobic microorganisms.

Summary and Explanation

Brewer[1] described a special Petri dish cover that allowed surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on an agar-based medium having a low oxidation-reduction potential. Anaerobic Agar is a modification of Brewer's original formula. This medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.[2-4]

Anaerobic bacteria cause a variety of infections in humans, including otitis media, oral infections, endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.[5,6] Anaerobic bacteria are the predominant flora colonizing the skin and mucous membranes of the body.[3] Anaerobes vary in their sensitivity to oxygen and nutritional requirements.[2] Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor.[3]

Principles of the Procedure

Peptone provides the nitrogen, vitamins and amino acids in Anaerobic Agar. Dextrose is a carbon source. Sodium chloride maintains the osmotic equilibrium. Sodium thioglycollate and sodium formaldehyde sulfoxylate are reducing agents. Methylene blue serves as an indicator of anaerobiosis with a blue color indicating the presence of oxygen. Agar is the solidifying agent.

Formula

Difco™ Anaerobic Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0	g
Sodium Chloride	5.0	g
Dextrose	10.0	g
Agar	20.0	g
Sodium Thioglycollate	2.0	g
Sodium Formaldehyde Sulfoxylate	1.0	g
Methylene Blue	2.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 58 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Standard Petri Dishes[2]

1. Inoculate a properly obtained specimen onto the medium using the pour plate technique.
2. Immediately incubate anaerobically at 35°C.
3. Examine at 24 hours if incubating plates in an anaerobic chamber. Examine at 48 hours if incubating plates in an anaerobic jar or anaerobic pouch.
4. Extended incubation may be necessary to recover some anaerobes.

User Quality Control

Identity Specifications

Difco™ Anaerobic Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent when hot, changing to green when cooled.
Prepared Appearance:	Light green, slightly opalescent.
Reaction of 5.8% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Anaerobic Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C under anaerobic conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	25285	10^2-10^3	Good
Clostridium perfringens	13124	10^2-10^3	Good
Fusobacterium mortiferum	9817	10^2-10^3	Good

Brewer Anaerobic Agar Plates

1. Dispense 50-60 mL of Anaerobic Agar into a standard Petri dish. For best results use porous tops to obtain a dry surface.
2. Inoculate the surface of the medium by streaking; avoid the edges of the plates.
3. Replace the standard Petri dish lid with a sterile Brewer anaerobic Petri dish cover. The cover should not rest on the Petri dish bottom. The inner glass ridge should seal against the uninoculated periphery of the agar. It is essential that the sealing ring inside the cover is in contact with the medium. This seal must not be broken before the end of the incubation period. A small amount of air is caught over the surface of the medium; however, the oxygen in this space reacts with reducing agents in the medium to form an anaerobic environment.
4. Incubate aerobically as desired.

For a complete discussion on anaerobic and microaerophilic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[2-5] For the examination of anaerobic bacteria in food, refer to standard methods.[7-9]

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[2]
2. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[2]
3. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure that the organism is an anaerobe.[2]
4. Methylene blue is toxic to some anaerobic bacteria.

References

1. Brewer. 1942. Science *95*:587.
2. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
3. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, Mo.
4. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
5. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.). Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
6. Smith. 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
7. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
9. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Anaerobic Agar

Cat. No. 253610 Dehydrated – 500 g

Antibiotic Assay Media
Antibiotic Medium 1 • Antibiotic Medium 2
Antibiotic Medium 3 • Antibiotic Medium 4
Antibiotic Medium 5 • Antibiotic Medium 8
Antibiotic Medium 9 • Antibiotic Medium 10
Antibiotic Medium 11 • Antibiotic Medium 12
Antibiotic Medium 13 • Antibiotic Medium 19

Intended Use

Antibiotic Assay Media are used for determining antibiotic potency by the microbiological assay technique.[1–3]

These media, where noted, meet *United States Pharmacopeia* (*USP*) performance specifications.

Summary and Explanation

The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.[2] Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.[2] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al.[4] for the assay of penicillin, was later modified by Foster and Woodruff[5] and by Schmidt and Moyer.[6]

Antibiotic assay media are prepared according to the specifications of the *USP*[2], European Pharmacopeia[7] and AOAC International.[3] The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.[1] Antibiotic Medium 19 corresponds to the use described in *Outline of Details for Official Microbiological Assays of Antibiotics*.[8]

Antibiotic Medium 12 is prepared from the Grove and Randall formula.[1] They recommended its use for preparing test plates for the cylinder plate assay of the antifungal agents, nystatin and anisomycin, using only a seed layer containing *Saccharomyces cerevisiae* as the test organism. It is used for the assay of amphotericin B.

Antibiotic Medium 1 and Antibiotic Medium 4 are used in a cylinder plate method for detecting penicillin in nonfat dry milk.[9]

The use of standardized culture media and careful control of all test conditions are fundamental requisites in the microbiological assay of antibiotics in order to achieve satisfactory test results.

Principles of the Procedure

Cylinder Plate Assay

This method is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. The diameter of a zone of inhibition after incubation depends, in part, on the concentration or activity of the antibiotic. This method is used in the assay of commercial preparations of antibiotics, as well as in the quantitative determination of antibiotics in body fluids, animal feeds and other materials.

Turbidimetric Assay

The turbidimetric method is based on the inhibition of growth of a microbial culture in a fluid medium containing a uniform solution of an antibiotic.[2] Turbidimetric determinations have the advantage of requiring a short incubation period, providing test results after 3 or 4 hours. However, the presence of solvents or other inhibitory materials may influence turbidimetric assays more markedly than cylinder plate assays. Use of this method is appropriate only when test samples are clear.

Formulae

Difco™ Antibiotic Medium 1 (Penassay Seed Agar)

Approximate Formula* Per Liter		
Beef Extract	1.5	g
Yeast Extract	3.0	g
Pancreatic Digest of Casein	4.0	g
Peptone	6.0	g
Dextrose	1.0	g
Agar	15.0	g

Difco™ Antibiotic Medium 2 (Penassay Base Agar)

Approximate Formula* Per Liter		
Beef Extract	1.5	g
Yeast Extract	3.0	g
Peptone	6.0	g
Agar	15.0	g

Difco™ Antibiotic Medium 3 (Penassay Broth)

Approximate Formula* Per Liter		
Beef Extract	1.5	g
Yeast Extract	1.5	g
Peptone	5.0	g
Dextrose	1.0	g
Sodium Chloride	3.5	g
Dipotassium Phosphate	3.68	g
Monopotassium Phosphate	1.32	g

Also Known As

DIFCO™ BRAND PRODUCT NAME	ALTERNATIVE DIFCO™ NAME	BBL™ BRAND PRODUCT NAME	*USP*[2]	AOAC[3]
Antibiotic Medium 1	Penassay Seed Agar	–	Medium 1	Agar Medium A
Antibiotic Medium 2	Penassay Base Agar	–	Medium 2	Agar Medium C
Antibiotic Medium 3	Penassay Broth	Antibiotic Assay Broth	Medium 3	Broth Medium A
Antibiotic Medium 4	Yeast Beef Agar	–	Medium 4	Agar Medium B
Antibiotic Medium 5	Streptomycin Assay Agar	–	Medium 5	Agar Medium E
Antibiotic Medium 8	–	–	Medium 8	Agar Medium D
Antibiotic Medium 9	Polymyxin Base Agar	–	Medium 9	–
Antibiotic Medium 10	Polymyxin Seed Agar	–	Medium 10	–
Antibiotic Medium 11	Neomycin Assay Agar	–	Medium 11	Agar Medium J
Antibiotic Medium 12	–	–	–	–
–	–	Sabouraud Liquid Broth, Modified	Medium 13	Broth Medium B
Antibiotic Medium 19	–	–	Medium 19	–

User Quality Control

Identity Specifications

	DEHYDRATED APPEARANCE	SOLUTION	PREPARED APPEARANCE	PH AT 25° C
Difco™Antibiotic Medium 1	Beige, homogeneous, free-flowing.	3.05% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent.	Light to medium amber, slightly opalescent.	6.55 ± 0.05
Difco™Antibiotic Medium 2	Light tan, homogeneous, free-flowing.	2.55% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.	Light-medium amber, slightly opalescent.	6.55 ± 0.05
Difco™Antibiotic Medium 3	Tan, free-flowing, homogeneous.	1.75% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear.	Light to medium amber, clear.	7.0 ± 0.05
BBL™Antibiotic Assay Broth (Antibiotic Medium 3)	Fine, homogeneous, free of extraneous material.	1.75% solution, soluble in purified water upon boiling. Solution is very pale to light, yellow to tan, clear to slightly hazy.	Pale to light, yellow to tan, clear to slightly hazy.	7.0 ± 0.2
Difco™Antibiotic Medium 4	Light tan, free-flowing, homogeneous.	2.65% solution, soluble in purified water upon boiling. Solution is light amber, very slightly opalescent.	Light amber, very slightly to slightly opalescent.	6.55 ± 0.05
Difco™Antibiotic Medium 5	Light tan, free-flowing, homogeneous.	2.55% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.	Light to medium amber, slightly opalescent.	7.9 ± 0.1
Difco™Antibiotic Medium 8	Light tan, free-flowing, homogeneous.	2.55% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.	Light to medium amber, slightly opalescent.	5.85 ± 0.05
Difco™Antibiotic Medium 9	Light beige, free-flowing, homogeneous.	5.0% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent, may have a slight flocculent precipitate.	Light to medium amber, slightly opalescent with slight flocculent precipitate.	7.25 ± 0.05
Difco™Antibiotic Medium 10	Beige, homogeneous, moist with a tendency to clump.	5.2% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.	Light to medium amber, slightly opalescent.	7.25 ± 0.05
Difco™Antibiotic Medium 11	Beige, homogeneous, free-flowing.	3.05% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.	Light to medium amber, slightly opalescent.	7.95 ± 0.05
Difco™Antibiotic Medium 12	Tan, homogeneous, free-flowing.	6.25% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.	Light to medium amber, slightly opalescent.	6.1 ± 0.1
BBL™Sabouraud Liquid Broth, Modified (Antibiotic Medium 13)	Fine, homogeneous, free of extraneous material.	3.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.	Light to medium, yellow to tan, clear to slightly hazy.	5.7 ± 0.1
Difco™Antibiotic Medium 19	Light tan, homogeneous, free-flowing.	6.0% solution, soluble in purified water upon boiling. Solution is medium amber, very slightly to slightly opalescent.	Medium amber, slightly opalescent.	6.1 ± 0.1

Continued

Cultural Response

Difco™ Antibiotic Medium 1
Difco™ Antibiotic Medium 2

Prepare the medium per label directions. Inoculate by the pour plate method and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Staphylococcus aureus	6538P	30-300	Good

Difco™ Antibiotic Medium 3

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for up to 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecium	10541	~10^7	Good
Escherichia coli	10536	~10^7	Good
Klebsiella pneumoniae	10031	~10^7	Good
Staphylococcus aureus	6538P	~10^7	Good

BBL™ Antibiotic Assay Broth (Antibiotic Medium 3)

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for the *Saccharomyces cerevisiae* and 35 ± 2°C for the remaining organisms for 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	≤ 10^3	Good
Escherichia coli	10536	≤ 10^3	Good
Kocuria rhizophila	9341	≤ 10^3	Good
Saccharomyces cerevisiae	9763	≤ 10^3	Good
Staphylococcus aureus	6538P	≤ 10^3	Good

Difco™ Antibiotic Medium 4

Prepare the medium per label directions. Inoculate by the pour plate method and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Kocuria rhizophila	9341	30-300	Good

Antibiotic Medium 5
Antibiotic Medium 8

Prepare the medium per label directions. Inoculate by the pour plate method and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	30-300	Good

Antibiotic Medium 9
Antibiotic Medium 10

Prepare the medium per label directions. Inoculate by the pour plate method and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bordetella bronchiseptica	4617	30-300	Good

Antibiotic Medium 11

Prepare the medium per label directions. Inoculate by the pour plate method and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Kocuria rhizophila	9341	30-300	Good
Staphylococcus epidermidis	12228	30-300	Good

Antibiotic Medium 12
Antibiotic Medium 19

Prepare the medium per label directions. Inoculate by the pour plate method and incubate at 30 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Saccharomyces cerevisiae	2601	30-300	Good

BBL™ Sabouraud Liquid Broth, Modified (Antibiotic Medium 13)

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for 7 days (use one loopful of a fresh 3-7 day culture for *A. brasiliensis* and *T. mentagrophytes*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	Undiluted	Good
Candida albicans	60193	≤ 10^3	Good
Saccharomyces cerevisiae	9763	≤ 10^3	Good
Trichophyton mentagrophytes	9533	Undiluted	Good

BBL™ Antibiotic Assay Broth (Antibiotic Medium 3)

Approximate Formula* Per Liter

Beef Extract	1.5	g
Yeast Extract	1.5	g
Pancreatic Digest of Gelatin	5.0	g
Dextrose	1.0	g
Sodium Chloride	3.5	g
Dipotassium Phosphate	3.68	g
Monopotassium Phosphate	1.32	g

Difco™ Antibiotic Medium 4 (Yeast Beef Agar)

Approximate Formula* Per Liter

Beef Extract	1.5	g
Yeast Extract	3.0	g
Peptone	6.0	g
Dextrose	1.0	g
Agar	15.0	g

Difco™ Antibiotic Medium 5 (Streptomycin Assay Agar)

Same as Medium 2, except for the final pH after autoclaving.

Difco™ Antibiotic Medium 8

Same as Medium 2, except for the final pH after autoclaving.

Difco™ Antibiotic Medium 9 (Polymyxin Base Agar)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Soy Peptone	3.0	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Agar	20.0	g

Difco™ Antibiotic Medium 10 (Polymyxin Seed Agar)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Soybean Peptone	3.0	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Agar	12.0	g
Polysorbate 80	10.0	g

Difco™ Antibiotic Medium 11 (Neomycin Assay Agar)

Same as Medium 1, except for the final pH after autoclaving.

Selection of Media for the Microbiological Assay of Antibiotics[2]

ANTIBIOTIC	ASSAY METHOD	ORGANISM	ATCC™	INOCULUM MEDIUM	CYLINDER PLATE BASE LAYER MEDIUM	CYLINDER PLATE SEED LAYER MEDIUM	TURBIDIMETRIC ASSAY MEDIUM
Amikacin	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Amphotericin B	Cylinder Plate	*Saccharomyces cerevisiae*	9763	19		19	
Bacitracin	Cylinder Plate	*Micrococcus luteus*	10240	1	2	1	
Candicidin	Turbidimetric	*Saccharomyces cerevisiae*	9763	19	2	13	
Capreomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1			3
Carbenicillin	Cylinder Plate	*Pseudomonas aeruginosa*	25619	1	9	10	
Cephalothin	Cylinder Plate	*Staphylococcus aureus*	29737	1	2	1	
Cephapirin	Cylinder Plate	*Staphylococcus aureus*	29737	1	2	1	
Chloramphenicol	Turbidimetric	*Escherichia coli*	10536	1			3
Chlortetracycline	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Cloxacillin	Cylinder Plate	*Staphylococcus aureus*	29737	1	2	1	
Colistimethate, sodium	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	9	10	
Colistin	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	9	10	
Cycloserine	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Demeclyocycline	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Dihydrostreptomycin	Cylinder Plate	*Bacillus subtilis*	6633	32*	5	5	
Dihydrostreptomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1			3
Doxycycline	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Erythromycin	Cylinder Plate	*Kocuria rhizophila*	9341	1	11	11	
Gentamicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	11	11	
Gramicidin	Turbidimetric	*Enterococcus hirae*	10541	3			3
Kanamycin	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Methacycline	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Nafcillin	Cylinder Plate	*Staphylococcus aureus*	29737	1	2	1	
Neomycin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	11	11	
Neomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1			39**
Netilmicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	11	11	
Novobiocin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	2	1	
Nystatin	Cylinder Plate	*Saccharomyces cerevisiae*	2601	19		19	
Oxytetracycline	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Paromomycin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	11	11	
Penicillin G	Cylinder Plate	*Staphylococcus aureus*	29737	1	2	1	
Polymyxin B	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	9	10	
Rolitetracycline	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Sisomicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	11	11	
Streptomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1			3
Tetracycline	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Tobramycin	Turbidimetric	*Staphylococcus aureus*	29737	1			3
Troleandomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1			3
Tylosin	Turbidimetric	*Staphylococcus aureus*	9144	3			39**
Vancomycin	Cylinder Plate	*Bacillus subtilis*	6633	32*	8	8	

** Same as Medium 1, except for the additional ingredient of 300 mg of manganese sulfate.*
*** Same as Medium 3, except that the final pH after autoclaving is 7.9 ± 0.1.*

Difco™ Antibiotic Medium 12

Approximate Formula* Per Liter

Beef Extract	2.5	g
Yeast Extract	5.0	g
Peptone	10.0	g
Dextrose	10.0	g
Sodium Chloride	10.0	g
Agar	25.0	g

BBL™ Sabouraud Liquid Broth, Modified (Antibiotic Medium 13)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	5.0	g
Peptic Digest of Animal Tissue	5.0	g
Dextrose	20.0	g

Difco™ Antibiotic Medium 19

Approximate Formula* Per Liter		
Beef Extract	2.4	g
Yeast Extract	4.7	g
Peptone	9.4	g
Dextrose	10.0	g
Sodium Chloride	10.0	g
Agar	23.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Antibiotic Medium 1 – 30.5 g;
   Difco™ Antibiotic Medium 2 – 25.5 g;
   Difco™ Antibiotic Medium 3 – 17.5 g;
   BBL™ Antibiotic Assay Broth (Antibiotic Medium 3) – 17.5 g;
   Difco™ Antibiotic Medium 4 – 26.5 g;
   Difco™ Antibiotic Medium 5 – 25.5 g;
   Difco™ Antibiotic Medium 8 – 25.5 g;
   Difco™ Antibiotic Medium 9 – 50 g;
   Difco™ Antibiotic Medium 10 – 52 g;
   Difco™ Antibiotic Medium 11 – 30.5 g;
   Difco™ Antibiotic Medium 12 – 62.5 g;
   BBL™ Sabouraud Liquid Broth, Modified (Antibiotic Medium 13) – 30 g;
   Difco™ Antibiotic Medium 19 – 60 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. To raise the pH of Antibiotic Medium 11 to 8.3 ± 0.1, cool the base to 45-50°C and add NaOH.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Test Organism Preparation

Maintain stock cultures on agar slants and make transfers at 1- or 2-week intervals. Prepare the inoculum for assay by washing growth from a fresh 24-48 hour agar slant using sterile purified water, saline or Antibiotic Medium 3 and further dilute the culture to obtain the desired organism concentration. In some turbidimetric assays, an 18- to 24-hour culture of the test organism in Antibiotic Medium 3, diluted to obtain the optimal number of organisms, is used.

When *Bacillus subtilis* is used as the test organism, inoculate it on Antibiotic Medium 1 and incubate at 37°C for 1 week, wash spores from the agar surface, and heat the spores at 56°C for 30 minutes. Wash the spores three times in purified water, heat again at 65°C for 30 minutes, and then dilute to the optimal concentration. This inoculum preparation should produce a sharp zone in the assay.

Antibiotic Medium 1 modified by the addition of 300 mg manganese sulfate ($MnSO_4 \bullet H_2O$) per liter often aids the sporulation of *B. subtilis* and may be used in preparing the spore suspension.

When *B. cereus* var. *mycoides* is required, inoculate the organism on Antibiotic Medium 1 and incubate at 30°C for 1 week. Wash and prepare the spores as for *B. subtilis*, above.

Cylinder Plate Assay

Use 20 × 100 mm glass or plastic Petri dishes with sufficient depth so that cylinders used in the assay will not be pushed into the medium by the cover.

Use stainless steel or porcelain assay cylinders having the following dimensions (± 0.1 mm): 8 mm outside diameter, 6 mm inside diameter and 10 mm long.[2] Carefully clean the cylinders to remove all residues, using an occasional acid bath (i.e., with approximately 2N nitric acid or with chromic acid).[2] Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius.

To assure accurate assays, work on a level surface to obtain uniformly thick base and seed layers in the Petri dish. Allow the base layer to solidify and then overlay the seed layer containing a proper concentration of the test organism. The amount of medium in the layers varies for different antibiotics, with most assays specifying a 21 mL base layer and a 4 mL seed layer. In any case, dishes with flat bottoms are required to assure complete coverage of the bottom of the dish when small amounts of base medium are used. Tilt the plate to obtain even coverage of the base layer by the seed layer and allow it to solidify in a level position. Plates should be used the same day as prepared.

Turbidimetric Assay

Use glass or plastic test tubes (i.e., 16 × 125 mm or 18 × 150 mm) that are relatively uniform in length, diameter and thickness and substantially free from surface blemishes.[2] Tubes that will be placed in the spectrophotometer should be matched and free of scratches or blemishes.[2] Clean the tubes thoroughly to remove all antibiotic residues and traces of cleaning solution and, prior to subsequent use, sterilize tubes that have been previously used.[2]

Prepare working dilutions of the antibiotic reference standards in specific concentrations. To a 1 mL quantity of each solution in a suitable tube, add 9 mL of inoculated broth, as required. Prepare similar solutions of the assay materials containing approximately the same amounts of antibiotic activity and place in tubes. Incubate the tubes for 3-4 hours at the required temperature, generally in a water bath. At the end of the incubation period, stop growth by adding 0.5 mL of 1:3 formalin. Determine the amount of growth by measuring light transmittance with a suitable spectrophotometer. Determine the concentration of the antibiotic by comparing the growth obtained with that given by reference standard solutions.

For a complete discussion of antibiotic assay methods, refer to appropriate procedures outlined in the references.[2,3,7]

Expected Results

Refer to appropriate procedures for results.[2,3,7]

References

1. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
2. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
4. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett *ii*:177.
5. Foster and Woodruff. 1943. J. Bacteriol. *46*:187.
6. Schmidt and Moyer. 1944. J. Bacteriol. *47*:199.
7. Council of Europe. 2002. European pharmacopeia, 4th ed. Council of Europe, Strasbourgh, France.
8. Kirshbaum and Arret. 1967. J. Pharm. Sci. *56*:512.
9. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Antibiotic Medium 1

AOAC EP USP

Cat. No. 226340 Dehydrated – 500 g

Difco™ Antibiotic Medium 2

AOAC USP

Cat. No. 227020 Dehydrated – 500 g

Difco™ Antibiotic Medium 3

AOAC EP USP

Cat. No. 224320 Dehydrated – 500 g
224310 Dehydrated – 2 kg

BBL™ Antibiotic Assay Broth (Antibiotic Medium 3)

AOAC EP USP

Cat. No. 210932 Dehydrated – 500 g

Difco™ Antibiotic Medium 4

AOAC USDA USP

Cat. No. 224410 Dehydrated – 500 g

Difco™ Antibiotic Medium 5

AOAC USDA USP

Cat. No. 227710 Dehydrated – 500 g

Difco™ Antibiotic Medium 8

AOAC USDA USP

Cat. No. 266710 Dehydrated – 500 g

Difco™ Antibiotic Medium 9

EP USP

Cat. No. 246210 Dehydrated – 500 g

Difco™ Antibiotic Medium 10

EP USP

Cat. No. 246310 Dehydrated – 500 g*

Difco™ Antibiotic Medium 11

AOAC USDA USP

Cat. No. 259310 Dehydrated – 500 g

Difco™ Antibiotic Medium 12

Cat. No. 266910 Dehydrated – 500 g

BBL™ Sabouraud Liquid Broth Modified (Antibiotic Medium 13)

AOAC USP

Cat. No. 210986 Dehydrated – 500 g
221014 Prepared Tubes (K Tubes) – Pkg. of 10

Difco™ Antibiotic Medium 19

EP USP

Cat. No. 243100 Dehydrated – 500 g

Europe

Cat. No. 254655 Prepared Bottles, 250 mL – Pkg. of 10

**Store at 2-8°C.*

Arylsulfatase Broth (0.001 M and 0.003 M)

Intended Use

Arylsulfatase Broths are chemically-defined media used in the differentiation of pathogenic mycobacteria based on their ability to produce arylsulfatase.

The 0.001 M Arylsulfatase Broth is used in a 3-day test to detect arylsulfatase activity in rapidly-growing mycobacteria. The 0.003 M Arylsulfatase Broth is used in a 14-day test for the detection of arylsulfatase in slow-growing mycobacteria.

Summary and Explanation

Arylsulfatase is produced by many mycobacterial species in varying concentrations.[1-3] The ability to produce a detectable level of arylsulfatase is a biochemical characteristic used in the differentiation of some *Mycobacterium* spp.[4-7]

The test is performed by inoculating a broth containing tripotassium phenolphthalein disulfate with a *Mycobacterium* isolate. If arylsulfatase is produced, it splits the phenolphthalein substrate, releasing free phenolphthalein, which turns pink to red when alkali is added to the medium.

The 0.001 M broth is used for rapidly-growing arylsulfatase producers, such as *M. fortuitum* and *M. chelonae*.[7]

The 0.003 M broth is used for slow-growing mycobacteria such as *M. flavescens, M. marinum, M. smegmatis, M. szulgai, M. trivale* and *M. xenopi*.[7]

Principles of the Procedure

Arylsulfatase Broth consists of Middlebrook 7H9 Broth supplemented with tripotassium phenolphthalein disulfate. Middlebrook 7H9 Broth is composed of inorganic compounds and albumin-dextrose enrichment to supply minerals and other nutrients necessary to support the growth of mycobacteria.

Tripotassium phenolphthalein disulfate is the substrate for the enzyme reaction. Arylsulfatase degrades the phenolphthalein substrate, producing free phenolphthalein, a pH indicator that becomes pink to red when the medium is made alkaline by the addition of sodium carbonate.

Precaution[8]

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety

Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[4] N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle, but effective, digesting and decontaminating agent. These reagents are provided in the **BBL™ MycoPrep™** Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[4-7]

Inoculate the broth with 0.1 mL of a 7-day liquid culture or heavily inoculate with organisms cultured on a solid medium.

Incubate the tubes at 35°C in an aerobic atmosphere without added CO_2. Remove the 0.001 M broth after 3 days and add no more than six drops of 1 M sodium carbonate solution (10.6 g anhydrous Na_2CO_3 in 100 mL of water), and observe for a color change.

Incubate the 0.003 M broth for 14 days, then remove and add six drops of the 1 M sodium carbonate solution. Observe for a color change.

Expected Results

A change in the color of the medium to pink or red following the addition of sodium carbonate is a positive reaction. The medium remains colorless if the reaction is negative.

References

1. Whitehead, Wildy and Engbaek. 1953. J. Pathol. Bacteriol. 65: 451.
2. Kubica and Vestal. 1961. Am. Rev. Respir. Dis. *83*: 728.
3. Kubica and Rigdon. 1961. Am. Rev. Respir. Dis. *83*: 737.
4. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.
5. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta, Ga.
6. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
7. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
8. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CD) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Arylsulfatase Broth (0.001 M)

Cat. No. 295654 Prepared Tubes – Pkg. of 10*

BBL™ Arylsulfatase Broth (0.003 M)

Cat. No. 297156 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*

Aspergillus Differential Agar

Intended Use

Aspergillus Differential Agar is used in the differentiation of *Aspergillus* species based on pigmentation.

Summary and Explanation

Bothast and Fennel developed Aspergillus Differential Agar as a screening medium to detect pigment produced under colonies of *Aspergillus flavus* (flavus group).[1] The yellow-orange pigment differentiates *A. flavus* from most other *Aspergillus* species and from organisms of other genera.[1-3] Some other *Aspergillus* species may also produce a yellow-orange pigment indistinguishable from the pigment produced by *A. flavus*.[1-3]

Principles of the Procedure

Aspergillus Differential Agar contains an enzymatic digest of casein to provide amino acids and other nitrogenous substances. Yeast extract primarily supplies the B-complex vitamins. Ferric citrate is essential for the production of a bright, yellow-orange pigment that differentiates *A. flavus* from most other clinically significant *Aspergillus* species.[1]

Procedure

The isolate to be differentiated should be stained with lactophenol cotton blue or an appropriate fungal stain and examined to confirm that morphology is appropriate for *Aspergillus* species. Using a sterile inoculating loop or needle, pick several isolated colonies and streak the surface of the slant. Incubate the tubes at 25°C for up to 10 days to allow sufficient time for pigmentation to develop.

Expected Results

Examine the medium for typical growth and pigmentation. *A. flavus* produces a yellow-orange pigment under colonies.

Limitation of the Procedure

A. parasiticus, another species associated with aspergillosis,[4] as well as some other aspergilli (i.e., *A. sulphureus, A. sclerotiorum* and *A. thomii*) may also produce a yellow-orange pigment that is indistinguishable from the pigment produced by *A. flavus*.[1,3]

References

1. Bothast and Fennel. 1974. Mycologia. 66:365.
2. Haley and Callaway. 1978. Laboratory methods in medical mycology, 4th ed. Center for Disease Control, Atlanta, Ga.
3. McGinnis. 1980. Laboratory handbook of medical mycology. Academic Press, New York, N.Y.
4. Kennedy and Sigler. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Aspergillus Differential Agar

Cat. No. 297244 Prepared Slants – Pkg. of 10

Azide Blood Agar Base

Intended Use

Azide Blood Agar Base is used for isolating streptococci and staphylococci and, supplemented with blood, for determining hemolytic reactions.

Summary and Explanation

In 1933, Edwards[1] used a liquid medium containing crystal violet and sodium azide as a selective broth in the isolation of mastitis streptococci. Snyder and Lichstein[2,3] reported that 0.01% sodium azide in blood agar prevented the swarming of *Proteus* species, and permitted the isolation of streptococci from mixed bacterial populations. Packer[4] modified Edwards' medium and prepared Infusion Blood Agar containing 1:15,000 sodium azide and 1:500,000 crystal violet for the study of bovine mastitis. Mallmann, Botwright and Churchill[5] reported that sodium azide exerted a bacteriostatic effect on gram-negative bacteria. The Azide Blood Agar Base formulation was based on the work of these researchers.

Azide Blood Agar Base is used in the isolation of gram-positive organisms from clinical and nonclinical specimens. Azide Blood Agar Base can be supplemented with 5-10% sheep, rabbit or horse blood for isolating, cultivating and determining hemolytic reactions of fastidious pathogens.

Principles of the Procedure

Peptones and beef extract provide nitrogen, vitamins, carbon and amino acids. Sodium chloride maintains osmotic balance. Sodium azide is the selective agent, suppressing the growth of gram-negative bacteria. Agar is the solidifying agent.

Supplementation with 5-10% blood provides additional growth factors for fastidious microorganisms, and is used to determine hemolytic patterns of bacteria.

Formula

Difco™ Azide Blood Agar Base

Approximate Formula* Per Liter		
Proteose Peptone No. 3	4.0	g
Pancreatic Digest of Casein	5.8	g
Beef Extract	3.0	g
Sodium Chloride	5.0	g
Sodium Azide	0.2	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 33 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium when cooled to 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Azide Blood Agar Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slight to slightly opalescent.
Prepared Appearance:	Plain–Light to medium amber, very slightly opalescent. With 5% blood–Cherry red, opaque.
Reaction of 3.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Azide Blood Agar Base

Prepare the medium per label directions, without and with 5% sterile defibrinated sheep blood. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
Enterococcus faecalis	19433	10^2-10^3	Good	Alpha/gamma
Escherichia coli	25922	10^3-2×10^3	Inhibition	–
Staphylococcus aureus	25923	10^2-10^3	Good	Beta
Staphylococcus epidermidis	12228	10^2-10^3	Good	Gamma
Streptococcus pneumoniae	6305	10^2-10^3	Good	Alpha
Streptococcus pyogenes	19615	10^2-10^3	Good	Beta

Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions demonstrating both oxygen-stable and oxygen-labile streptolysins.[6]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 in accordance with established laboratory procedures.

Expected Results

Examine plates for growth and hemolytic reactions after 18-24 and 40-48 hours of incubation. Four different types of hemolysis on blood agar media can be described:[7]

a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.
b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.

d. Alpha-prime (ά)-hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Azide Blood Agar Base is intended for selective use and should be inoculated in parallel with nonselective media.
2. Hemolytic patterns of streptococci grown on Azide Blood Agar Base are somewhat different than those observed on ordinary blood agar. Sodium azide enhances hemolysis. Alpha and beta zones may be extended.[4]
3. Hemolytic patterns may vary with the source of animal blood or base medium used.[6]

References

1. Edwards. 1933. J. Comp. Pathol. Therap. *46*:211.
2. Snyder and Lichstein. 1940. J. Infect. Dis. *67*:113.
3. Lichstein and Snyder. 1941. J. Bacteriol. *42*:653.
4. Packer. 1943. J. Infect. Dis. *67*:113.
5. Mallmann, Botwright and Churchill. 1943. J. Bacteriol. *46*:343.
6. Ruoff, Whiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Azide Blood Agar Base

Cat. No. 240920 Dehydrated – 500 g

Azide Dextrose Broth

Intended Use

Azide Dextrose Broth is used for cultivating streptococci in water and wastewater.

Summary and Explanation

The formula for Azide Dextrose Broth originated with Rothe at the Illinois State Health Department.[1] In a comparative study, Mallmann and Seligmann[2] investigated the detection of streptococci in water and wastewater using Azide Dextrose Broth. Their work supported use of the medium in determining the presence of streptococci in water, wastewater, shellfish and other materials. Azide Dextrose Broth has also been used for primary isolation of streptococci from foodstuffs[3,4] and other specimens of sanitary significance as an indication of fecal contamination.

Azide Dextrose Broth is specified for use in the presumptive test of water and wastewater for fecal streptococci by the Multiple-Tube Technique.[5]

Principles of the Procedure

Azide Dextrose Broth contains beef extract and peptones as sources of carbon, nitrogen, vitamins and minerals. Dextrose is a fermentable carbohydrate. Sodium chloride maintains the osmotic balance of the medium. Sodium azide inhibits cytochrome oxidase in gram-negative bacteria.

Group D streptococci grow in the presence of azide, ferment glucose and cause turbidity.

Formula

Difco™ Azide Dextrose Broth

Approximate Formula* Per Liter		
Beef Extract	4.5	g
Pancreatic Digest of Casein	7.5	g
Proteose Peptone No. 3	7.5	g
Dextrose	7.5	g
Sodium Chloride	7.5	g
Sodium Azide	0.2	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Azide Dextrose Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.47% (single strength) solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 3.47% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Azide Dextrose Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	19433	10^2-10^3	Good
Escherichia coli	25922	3×10^2-10^3	Inhibition

Uninoculated Tube

Enterococcus faecalis ATCC™ 29212

Directions for Preparation from Dehydrated Product

1. Dissolve 34.7 g of the powder in 1 L of purified water for the preparation of single-strength broth for inoculation of samples of 10 mL or smaller. Use 69.4 g for 1 L of double-strength broth for samples larger than 10 mL.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure[5]

1. Inoculate a series of Azide Dextrose Broth tubes with appropriately graduated quantities of sample. Use sample quantities of 10 mL or less. Use double-strength broth for 10 mL inocula. Consult an appropriate reference for suggested sample sizes.[5]
2. Incubate inoculated tubes at 35 ± 2°C for 20-48 hours.
3. Examine each tube for turbidity at the end of 24 ± 2 hours. If no turbidity is evident, reincubate and read again at the end of 48 ± 3 hours.

Expected Results

A positive test is indicated by turbidity (cloudiness) in the broth. A negative test remains clear.

All Azide Dextrose Broth tubes showing turbidity after 24- or 48-hours of incubation must be subjected to the Confirmed Test Procedure. Consult appropriate references for details of the Confirmed Test Procedure[5] and further identification of *Enterococcus*.[5,6]

Limitations of the Procedure

1. Azide Dextrose Broth is used to detect presumptive evidence of fecal contamination. Further biochemical testing must be done for confirmation.
2. For inoculum sizes of 10 mL or larger, use double strength medium to prevent dilution of ingredients.[5,6]

References

1. Rothe. 1948. Illinois State Health Department.
2. Mallmann and Seligmann. 1950. Am. J. Public Health *40*:286.
3. Larkin, Litsky and Fuller. 1955. Appl. Microbiol. 3:98.
4. Splittstoesser, Wright and Hucker. 1961. Appl. Microbiol. 9:303.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Azide Dextrose Broth

EPA SMWW

Cat. No. 238710 Dehydrated – 500 g

B_{12} Assay Medium

Intended Use

B_{12} Assay Medium is used for determining vitamin B_{12} concentration by the microbiological assay technique.

Meets *United States Pharmacopeia (USP)* performance specifications.

User Quality Control

Identity Specifications

Difco™ B_{12} Assay Medium

Dehydrated Appearance:	Very light to light beige, homogeneous, with a tendency to clump.
Solution:	4.25% (single strength) solution, soluble in purified water upon boiling for 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light to light amber, clear, may have a slight precipitate.
Reaction of 4.25% Solution at 25°C:	pH 6.0 ± 0.1

Cultural Response

Difco™ B_{12} Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus delbrueckii* subsp. *lactis* ATCC™ 7830 when prepared in single strength and supplemented with cyanocobalamin (vitamin B_{12}). The medium should produce a standard curve when tested with a cyanocobalamin reference standard at 0.0 to 0.25 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-24 hours. Read the percent transmittance using a spectrophotometer at 530 nm.

Summary and Explanation

Vitamin assay media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

B_{12} Assay Medium is used in the microbiological assay of vitamin B_{12} according to the procedures of the Vitamin B_{12} Activity Assay in the *USP*[1] and the Cobalamin (Vitamin B_{12} Activity) Assay in the *Official Methods of Analysis of AOAC International* (AOAC).[2] *Lactobacillus delbrueckii* subsp. *lactis* ATCC™ 7830 (*Lactobacillus leichmannii*) is the test organism used in this procedure.

Principles of the Procedure

B_{12} Assay Medium is a vitamin B_{12}-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. delbrueckii* subsp. *lactis* ATCC 7830. To obtain a standard curve, USP Cyanocobalamin Reference is added in specified increasing concentrations giving a growth response that can be measured titrimetrically or turbidimetrically.

Formula

Difco™ B_{12} Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	15.0	g
Dextrose	40.0	g
Asparagine	0.2	g
Sodium Acetate	20.0	g
Ascorbic Acid	4.0	g
L-Cystine	0.4	g
DL-Tryptophan	0.4	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Xanthine	20.0	mg
Riboflavin	1.0	mg
Thiamine Hydrochloride	1.0	mg
Biotin	10.0	µg
Niacin	2.0	mg
p-Aminobenzoic Acid	2.0	mg
Calcium Pantothenate	1.0	mg
Pyridoxine Hydrochloride	4.0	mg
Pyridoxal Hydrochloride	4.0	mg
Pyridoxamine Hydrochloride	800.0	µg
Folic Acid	200.0	µg
Monopotassium Phosphate	1.0	g
Dipotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg
Polysorbate 80	2.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 8.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense in 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the tube volume to 10 mL with purified water.
6. Autoclave at 121˚C for 5 minutes.

Procedure

Follow assay procedures as outlined in *USP*[1] or AOAC.[2] Use levels of B_{12} in the preparation of the standard curve according to these references. It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. Generally satisfactory results are obtained with B_{12} at the following levels: 0.0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 and 0.25 ng per assay tube (10 mL).

Stock cultures of *L. delbrueckii* subsp. *lactis* ATCC 7830 are prepared by stab inoculation into 10 mL of B_{12} Culture Agar or Lactobacilli Agar AOAC. After 16-24 hours incubation at 35-37°C, the cultures are kept refrigerated. The inoculum for assay is prepared by subculturing a stock culture of *L. delbrueckii* subsp. *lactis* into 10 mL of B_{12} Inoculum Broth. For a complete discussion on B_{12} Culture Agar and B_{12} Inoculum Broth, refer to *USP*.[1]

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average and use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. For successful results to these procedures, all conditions of the assay must be followed precisely.
3. Aseptic technique should be used throughout the assay procedure.
4. The use of altered or deficient media may cause mutants having different nutritional requirements and will not give a satisfactory response.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ B_{12} Assay Medium

AOAC USP

Cat. No. 245710 Dehydrated – 100 g*

**Store at 2-8˚C.*

B_{12} Culture Agar • B_{12} Inoculum Broth

Intended Use

B_{12} Culture Agar is used for cultivating *Lactobacillus delbrueckii* subsp. *lactis* ATCC™ 7830 used in the Vitamin B_{12} Activity Assay.

B_{12} Inoculum Broth is used for preparing the inoculum of *L. delbrueckii* subsp. *lactis* ATCC 7830 used in the Vitamin B_{12} Activity Assay.

These media meet *United States Pharmacopeia (USP)* performance specifications.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

User Quality Control

Identity Specifications

Difco™ B_{12} Culture Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in purified water upon boiling. Solution is light to medium amber, opalescent when hot, slightly opalescent with flocculent precipitate when cooled.
Prepared Appearance:	Light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Reaction of 4.7% Solution at 25°C:	pH 6.8 ± 0.1

Difco™ B_{12} Inoculum Broth

Dehydrated Appearance:	Tan, homogeneous, tendency to clump.
Solution:	3.2% solution, soluble in purified water upon boiling. Solution is medium to dark amber, opalescent when hot, clear when cooled to room temperature.
Prepared Appearance:	Medium amber, clear.
Reaction of 3.2% Solution at 25°C:	pH 6.8 ± 0.1

Cultural Response

Difco™ B_{12} Culture Agar or B_{12} Inoculum Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 16-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus delbrueckii subsp. *lactis*	7830	3×10^2 -10^3	Good

Lactobacillus species grow poorly on nonselective culture media and require special nutrients. Mickle and Breed[2] reported the use of tomato juice in culture media for lactobacilli. Kulp,[3] while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.

B_{12} Culture Agar is recommended for maintaining stock cultures of *L. delbrueckii* subsp. *lactis* ATCC 7830 (*Lactobacillus leichmannii*) for use in the Vitamin B_{12} Activity Assay according to the *USP*.[1]

B_{12} Inoculum Broth is used for preparing the inoculum of *L. delbrueckii* subsp. *lactis* ATCC 7830 in the microbiological assay of vitamin B_{12} according to the *USP*.[1]

Principles of the Procedure

Peptone provides the nitrogen and amino acids in B_{12} Culture Agar and B_{12} Inoculum Broth. Yeast extract is the vitamin source in the formulas. Tomato juice is added to create the proper acidic environment. Dextrose is the carbon source, and polysorbate 80 acts as an emulsifier. Dipotassium phosphate acts as the buffering agent in B_{12} Inoculum Broth, and monopotassium phosphate is the buffering agent in B_{12} Culture Agar. Agar is the solidifying agent in B_{12} Culture Agar.

Formulae

Difco™ B_{12} Culture Agar

Approximate Formula* Per Liter		
Tomato Juice (from 100 mL)	5.0	g
Proteose Peptone No. 3	7.5	g
Yeast Extract	7.5	g
Dextrose	10.0	g
Monopotassium Phosphate	2.0	g
Polysorbate 80	1.0	g
Agar	14.0	g

Difco™ B_{12} Inoculum Broth

Approximate Formula* Per Liter		
Tomato Juice (from 100 mL)	5.0	g
Proteose Peptone No. 3	7.5	g
Yeast Extract	7.5	g
Dextrose	10.0	g
Dipotassium Phosphate	2.0	g
Polysorbate 80	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used.

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ B_{12} Culture Agar – 47 g;
   Difco™ B_{12} Inoculum Broth – 32 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense 10 mL amounts into tubes.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion of vitamin assay methodology, refer to appropriate procedures outlined in the *USP*.[1]

Expected Results

For test results of vitamin assay procedures refer to the *USP*.[1]

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. For successful results of these procedures, all conditions of the assay must be followed precisely.
3. Aseptic technique should be used throughout the assay procedure.
4. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 1-8-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Mickle and Breed. 1925. Technical Bulletin 110, NY State Agriculture Ex. Station, Geneva, N.Y.
3. Kulp and White. 1932. Science 76:17.

Availability

Difco™ B_{12} Culture Agar

USP

Cat. No. 254110 Dehydrated – 100 g*

Difco™ B_{12} Inoculum Broth

USP

Cat. No. 254210 Dehydrated – 100 g*

**Store at 2-8°C.*

BCYE Agars
BCYE Agar Base • BCYE Agar • BCYE Differential Agar BCYE Selective Agars (CCVC, PAC, PAV) • Legionella Agar Base • Legionella Agar Enrichment

Intended Use

These media are used in qualitative procedures for isolation of *Legionella* species from clinical specimens and nonclinical (environmental) samples.

Summary and Explanation

BCYE Agar is based on Edelstein's modification of previously described media. In 1979, Feely et al. described Charcoal Yeast Extract (CYE) Agar as a modification of an existing medium, F-G Agar.[1,2] They replaced the starch in the F-G Agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in better recovery of *L. pneumophila*. In 1980, Pasculle reported that CYE Agar could be improved by buffering the medium with ACES Buffer.[3] A year later, Edelstein further increased the sensitivity of the medium by adding alpha-ketoglutarate (BCYE Agar).[4]

Legionella Agar is a modification of the BCYE Agar formula of Edelstein. In the formula, the concentration of ACES buffer was reduced from 10.0 g/L to 6.0 g/L.

BCYE Differential Agar is used for the presumptive identification and differentiation of *Legionella* spp. based on colony morphology and color.[5] This medium is based on the formulation of Vickers et al.,[6] and consists of the dyes bromcresol purple and bromthymol blue added to BCYE Agar.

BCYE Selective Agar w/CCVC is a highly selective medium consisting of BYCE Agar supplemented with cephalothin, colistin, vancomycin and cycloheximide. This medium is based on the formulation of Bopp et al.[7] They obtained improved recovery of *L. pneumophila* by using the selective medium in conjunction with an acid wash treatment to reduce the contaminating microbial flora present in environmental water samples.

BCYE Selective Agar with PAC was developed by Edelstein for isolation of *Legionella* spp. from specimens containing mixed flora.[4] He found that BYCE Agar supplemented with polymyxin B, cefamandole and anisomycin enhanced the recovery of *L. pneumophila* from contaminated clinical specimens. In conjunction with an acid wash treatment to reduce microbial flora, it also facilitated the recovery of the bacterium from potable water.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Legionella Agar Base

Dehydrated Appearance:	Dark gray, free flowing, homogeneous.
Solution:	3.7% solution, insoluble in purified water. Suspension is black, opalescent.
Prepared Appearance:	Black, opaque with precipitate.
Reaction of 3.7% Solution at 25°C:	pH 6.85-7.0 (adjusted)

Difco™ Legionella Agar Enrichment

Lyophilized Appearance:	White to off-white powder, with or without traces of brown or green powder.
Solution:	After rehydration, white to off-white, opaque, milky suspension. Some evidence of browning may be present due to ferric pyrophosphate.

Cultural Response

Difco™ Legionella Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under a humidified atmosphere containing approximately 2.5% CO_2 for 46-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Legionella dumoffii	33279	30-300	Good	Gray to off-white
Legionella pneumophila	33153	30-300	Good	Gray to off-white
Legionella pneumophila	33154	30-300	Good	Gray to off-white (may have a blue cast)

Identity Specifications

BBL™ BCYE Agar Base

Dehydrated Appearance:	Fine to coarse, homogeneous, free of extraneous material.
Solution:	3.83% solution, soluble in purified water upon boiling. Solution is dark, black-green to gray-black, moderately hazy to opaque.
Prepared Appearance:	Dark, black-green to gray-black, moderately hazy to opaque.
Reaction of 3.83% Solution at 25°C:	pH 6.85 ± 0.1 (adjusted)

Cultural Response

BBL™ BCYE Agar Base

Prepare the medium per label directions. For *E. coli*, inoculate and incubate at 35 ± 2°C for 66-72 hours. For *Legionella* spp., inoculate test and control lots of medium with serial 10-fold dilutions of the test organisms and incubate at 35 ± 2°C for 66-72 hours. Growth on the test lot should be within 1 log of the control lot.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	FLUORESCENCE
Escherichia coli	25922	10^3-10^4	Good	–
Legionella bozemanii	33217	N/A	Good (white-gray to blue-gray colonies)	Blue-white
Legionella pneumophila	33152	N/A	Good	Yellow green

Legionella pneumophila
ATCC™ 33152

BCYE Selective Agar with PAV is similar to the Edelstein formula, above, except that the concentration of polymyxin B is reduced by half, and vancomycin is substituted for cefamandole.

Principles of the Procedure

These media consist of a base medium (BCYE) supplemented with antibiotics or dyes. Antibiotics improve the recovery of *Legionella* spp. by inhibiting the growth of contaminating organisms. Dyes facilitate differentiation and identification of *Legionella* spp.

The base media (BCYE Agar Base and Legionella Agar Base) contain yeast extract to supply the nutrients necessary to support bacterial growth. L-cysteine HCl, ferric pyrophosphate and alpha-ketoglutarate are incorporated to satisfy the specific nutritional requirements of *Legionella* species. The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect carbon dioxide and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth of *Legionella* species.

Antibiotics incorporated in the various BCYE formulations have different spectra of activity. Vancomycin inhibits gram-positive bacteria; colistin and polymyxin B inhibit gram-negative bacteria, except for *Proteus* spp.; and cephalothin and cefamandole inhibit both gram-positive and gram-negative bacteria. Anisomycin and cycloheximide are antifungal agents.

BCYE Differential Agar contains the dyes bromcresol purple and bromthymol blue to aid in the differentiation and identification of *Legionella* species.

Formulae

Difco™ Legionella Agar Base

Approximate Formula* Per Liter

Yeast Extract	11.5	g
ACES Buffer	6.0	g
Charcoal, Activated	1.5	g
Alpha-Ketoglutarate, Monopotassium	1.0	g
Agar	17.0	g

Difco™ Legionella Agar Enrichment

Approximate Formula* Per Vial

L-Cysteine HCl	0.35	g
Ferric Pyrophosphate	0.14	g

BBL™ BCYE Agar Base

Approximate Formula* Per Liter

Yeast Extract	10.0	g
Ferric Pyrophosphate	0.25	g
ACES Buffer	10.0	g
Charcoal, Activated	2.0	g
Alpha-Ketoglutarate	1.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Legionella Agar Base

1. Dissolve 18.5 g of the powder in 500 mL of purified water.
2. Adjust to pH 7.1-7.2 with 1 N KOH. Do not heat prior to autoclaving.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C and aseptically add 5 mL rehydrated Difco™ Legionella Agar Enrichment. Mix thoroughly.
5. Check pH. If necessary, aseptically adjust to pH 6.85-7.0 with 1 N HCl or 1 N KOH.
6. Dispense into Petri dishes. Agitate while dispensing to keep charcoal in suspension.
7. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ BCYE Agar Base

1. To 500 mL of purified water, add 2.4 g KOH pellets and mix to dissolve.
2. Add 38.3 g of the powder and 500 mL of purified water. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45-50°C and add 4 mL of a 10% filter-sterilized solution of L-cysteine HCl.
6. Mix thoroughly. Check pH; if not 6.85 ± 0.1, adjust using 1 N HCl or KOH.
7. Dispense into Petri dishes. Agitate while dispensing to keep charcoal in suspension.
8. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens and samples.

Incubate the plates in an inverted position (agar side up) at 35 ± 2°C for a minimum of 3 days. Growth is usually visible within 3-4 days, but may take up to 2 weeks to appear.

Expected Results

On BCYE Agar, Legionella Agar and the selective media, *Legionella pneumophila* produces small to large, smooth, colorless to pale, blue-gray, slightly mucoid colonies that fluoresce yellow-green under long-wave UV light. Consult references for morphology and color of fluorescence of other species.[8,9]

On BCYE Differential Agar, *L. pneumophila* produces light blue colonies with a pale green tint. *L. micdadei* produces blue-gray to dark blue colonies.

A Gram stain, biochemical tests and serological procedures should be performed to confirm findings.

References

1. Feely, Gibson, Gorman, Langford, Rasheed, Mackel and Baine. 1979. J. Clin. Microbiol. *10*:437.
2. Feely, Gorman, Weaver, Mackel and Smith. 1978. J. Clin. Microbiol. *8*:320.
3. Pasculle, Feely, Gibson, Cordes, Myerowitz, Patton, Gorman, Carmack, Ezzell and Dowling. 1980. J. Infect. Dis. *191*:727.
4. Edelstein. 1981. J. Clin. Microbiol. *14*:298.
5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
6. Vickers, Brown and Garrity. 1981. J. Clin. Microbiol. *13*:380.
7. Bopp, Sumner, Morris and Wells. 1981. J. Clin. Microbiol. *13*:714.
8. Edelstein. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
9. Weaver. 1978. *In* Jones and Herbert (ed.), "Legionnaires": the disease, the bacterium and methodology. DHEW, Center for Disease Control, Atlanta, Ga.

Availability

Difco™ Legionella Agar Base

Cat. No. 218301 Dehydrated – 500 g

Difco™ Legionella Agar Enrichment

Cat. No. 233901 Vial, 5 mL – Pkg. of 6*

BBL™ BCYE Agar Base

SMWW

Cat. No. 212327 Dehydrated – 500 g

BBL™ BCYE Agar

BS12 CMPH2 MCM9

United States and Canada

Cat. No. 221808 Prepared Plates – Pkg. of 10*

Europe

Cat. No. 257321 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 252164 Prepared Plates – Pkg. of 20*

BBL™ BCYE Differential Agar

Cat. No. 297881 Prepared Plates – Pkg. of 10*

BBL™ BCYE Selective Agar with PAC

BS12 CMPH2 MCM9

Cat. No. 297879 Prepared Plates – Pkg. of 10*

BBL™ BCYE Selective Agar with PAV

BS12 MCM9

Cat. No. 297880 Prepared Plates – Pkg. of 10*

BBL™ BCYE Selective Agar with CCVC

Cat. No. 297878 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

BG Sulfa Agar
SBG Sulfa Enrichment

Intended Use

BG Sulfa Agar is used for isolating *Salmonella*.

SBG Sulfa Enrichment is used for enriching *Salmonella* prior to isolation procedures.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. The illness results from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many of these cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. Various poultry products are routinely monitored for *Salmonella* before their distribution for human consumption, but in many instances, contaminated food samples elude detection.

BG (Brilliant Green) Sulfa Agar is a highly selective medium. Osborne and Stokes[1] added 0.1% sodium sulfapyridine to Brilliant Green Agar to enhance the selective properties of this medium for *Salmonella*. This formula is recommended as a selective isolation medium for *Salmonella* following enrichment.[2] It is also recommended for direct inoculation with primary specimens for *Salmonella* isolation.

For food testing, BG Sulfa Agar has been used for detection of *Salmonella* in low and high moisture foods.[3,4] It has also been used for detecting *Salmonella* in feeds and feed ingredients.[5] This medium is recommended when testing foods for *Salmonella* following USDA guidelines.[6-8]

SBG (Selenite Brilliant Green) Sulfa Enrichment is prepared according to the formula described by Stokes and Osborne.[9] The researchers found that whole egg and egg yolk reduced the selective properties of selenite brilliant green enrichment.[1] They also found that the addition of sulfapyridine (SBG Sulfa Enrichment) restored these selective properties.[1]

SBG Sulfa Enrichment is a selective enrichment for the isolation of *Salmonella* species, especially from egg products. The shell and the contents of the egg at the time of oviposition are generally sterile or harbor very few microorganisms. Contamination of the shell occurs afterwards from nesting material, floor litter and avian fecal matter.[10-12]

Principles of the Procedure

In BG Sulfa Agar, peptone and yeast extract provide nitrogen, vitamins and minerals. Lactose and sucrose are the sources of carbohydrates in the medium. Brilliant green and sodium pyridine are complementary in inhibiting gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Phenol red is the pH indicator that turns the medium a yellow color with the formation of acid when lactose and/or sucrose is fermented. Agar is the solidifying agent.

Peptone provides the nitrogen, minerals and amino acids in SBG Sulfa Enrichment. Yeast extract is the vitamin source. D-Mannitol is the carbon source to stimulate organism growth. The phosphates act as buffers in the enrichment. Sodium taurocholate, sodium selenite and brilliant green are the selective agents. The selective agents are used to inhibit gram-positive organisms and enteric bacteria other than *Salmonella*. Sodium sulfapyridine is added to increase selectivity.

Formulae

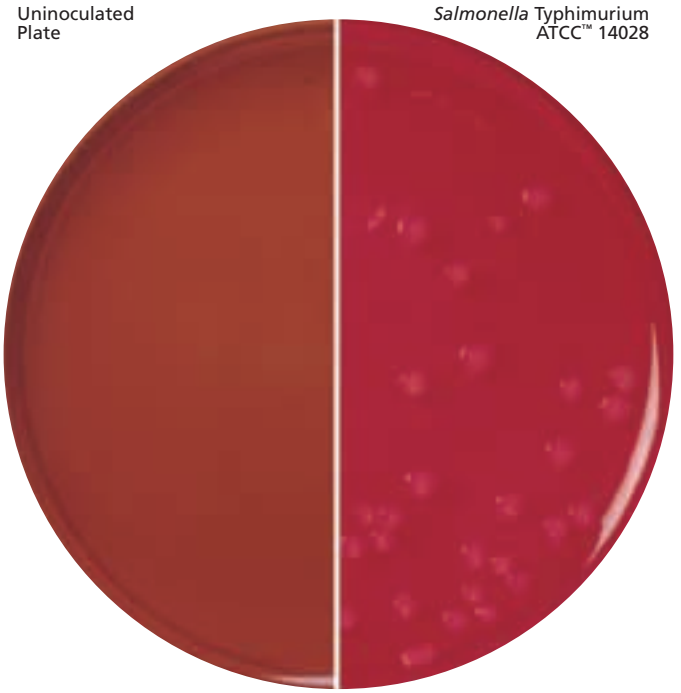
Difco™ BG Sulfa Agar

Approximate Formula* Per Liter		
Yeast Extract	3.0	g
Proteose Peptone No. 3	10.0	g
Lactose	10.0	g
Saccharose	10.0	g
Sodium Sulfapyridine	1.0	g
Sodium Chloride	5.0	g
Agar	20.0	g
Brilliant Green	12.5	mg
Phenol Red	0.08	g

Difco™ SBG Sulfa Enrichment

Approximate Formula* Per Liter		
Yeast Extract	5.0	g
Peptone	5.0	g
D-Mannitol	5.0	g
Sodium Taurocholate	1.0	g
Sodium Sulfapyridine	0.5	g
Sodium Selenite	4.0	g
Dipotassium Phosphate	2.65	g
Monopotassium Phosphate	1.02	g
Brilliant Green	5.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*



User Quality Control

Identity Specifications
Difco™ BG Sulfa Agar

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	5.9% solution, soluble in purified water upon boiling. Solution is very dark amber, very slightly to slightly opalescent.
Prepared Appearance:	Orange-brown to dark reddish-amber, slightly opalescent.
Reaction of 5.9% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response
Difco™ BG Sulfa Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF COLONIES/ MEDIUM
Enterococcus faecalis	29212	10^3-2×10^3	None	–/no change
Escherichia coli	25922	10^2-3×10^2	None to poor	Yellow-green/ Yellow-green
Proteus vulgaris	13315	10^2-3×10^2	None	–/no change
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10^2-3×10^2	Good	Pink-white/ red
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-3×10^2	Good	Pink-white/ red

Identity Specifications
Difco™ SBG Sulfa Enrichment

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.42% solution, soluble in purified water. Solution is green, opalescent, may have a precipitate.
Prepared Appearance:	Green, opalescent without significant precipitate.
Reaction of 2.42% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response
Difco™ SBG Sulfa Enrichment

Prepare the enrichment per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, subculture onto plates of MacConkey Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR ON MACCONKEY
Escherichia coli	25922	10^2-3×10^2	None to poor	Pink, if any
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10^2-3×10^2	Good	Colorless
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-3×10^2	Good	Colorless
Shigella sonnei	9290	10^2-3×10^2	None to fair	Colorless

Directions for Preparation from Dehydrated Product
Difco™ BG Sulfa Agar

1. Suspend 59 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating, which will decrease selectivity.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ SBG Sulfa Enrichment

1. Suspend 24.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 5-10 minutes to completely dissolve the powder. Avoid overheating. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Refer to appropriate references for specific procedures for the isolation and cultivation of *Salmonella* from meat, poultry and egg products and other foods.[2,7,8]

Expected Results
BG Sulfa Agar

The typical *Salmonella* colonies appear as pink-white to red opaque colonies surrounded by a brilliant red medium. The few lactose and/or sucrose fermenting organisms that grow are readily differentiated due to the formation of a yellow-green colony surrounded by an intense yellow-green zone. BG Sulfa Agar is not suitable for the isolation of *S.* Typhi or *Shigella*; however, some strains of *S.* Typhi may grow forming red colonies.

SBG Sulfa Enrichment

Examine prepared media for growth. Positive tubes should be subcultured onto prepared media for isolation and identification of bacteria.

Limitations of the Procedure
1. On BG Sulfa Agar colonies of *Salmonella* spp. vary from red to pink to white depending on length of incubation and strain.[13]
2. BG Sulfa Agar is normally orange-brown in color; however, on incubation, it turns bright red and returns to normal color at room temperature.[13]
3. *S.* Typhi does not grow adequately on BG Sulfa Agar. *Shigella* spp. do not grow on BG Sulfa Agar.[13]
4. Do not autoclave BG Sulfa Agar longer than 15 minutes; longer periods decrease the selectivity of the medium.

5. Since BG Sulfa Agar is highly selective, it is recommended that less selective media, such as MacConkey Agar, be used simultaneously.
6. SBG Sulfa Enrichment should be used in conjunction with a selective prepared medium for bacterial identification.

References

1. Osborn and Stokes. 1955. Appl. Microbiol. *3*:295.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. D'Aoust, Maishment, Burgener, Conley, Loit, Milling and Purvis. 1980. J. Food Prot. *43*:343.
4. D'Aoust. 1984. J. Food Prot. *47*:588.
5. D'Aoust, Sewell and Boville. 1983. J. Food Prot. *46*:851.
6. Moats. 1981. J. Food Prot. *44*:375.
7. Federal Register. 1996. Fed. Regist. *61*:38917.
8. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.
9. Osborn and Stokes. 1955. Appl. Microbiol. *3*:217.
10. Brooks and Taylor. 1955. Rep. Rd. Invest., Bd. 60, H. M. S. O. London, England.
11. Forsythe, Ayres and Radlo. 1953. Food Technol. 7:49.
12. Stadelman, Ikeme, Roop and Simmons. 1982. Poultry Sci. *61*:388.
13. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ BG Sulfa Agar

CCAM COMPF USDA

Cat. No. 271710 Dehydrated – 500 g

Difco™ SBG Sulfa Enrichment

USDA

Cat. No. 271510 Dehydrated – 500 g

BiGGY Agar

Intended Use

BiGGY (Bismuth Sulfite Glucose Glycine Yeast) is a selective and differential medium used in the detection, isolation and presumptive identification of *Candida* species.

Summary and Explanation

BiGGY Agar is based on the formulation of Nickerson.[1] Nickerson developed the medium in 1953 following a study of sulfite reduction by *Candida* species.

Differentiation of *Candida* is based on growth patterns and pigmentation of isolated colonies. The bismuth sulfite acts as an inhibitory agent to suppress bacterial growth, which enables the recovery of isolated colonies of *Candida*. *Candida* species reduce the bismuth sulfite, resulting in pigmentation of colonies and, with some species, pigmentation in the surrounding medium.

Principles of the Procedure

Candida species, through a process of substrate reduction, produce sulfide and bismuth which combine to produce brown to black pigmented colonies and zones of dark precipitate in the medium surrounding colonies of some species. Dextrose and yeast extract provide the nutrients in the formulation.

NOTE: A decrease in pH is normal and does not affect performance.

User Quality Control

Identity Specifications

BBL™ BiGGY Agar

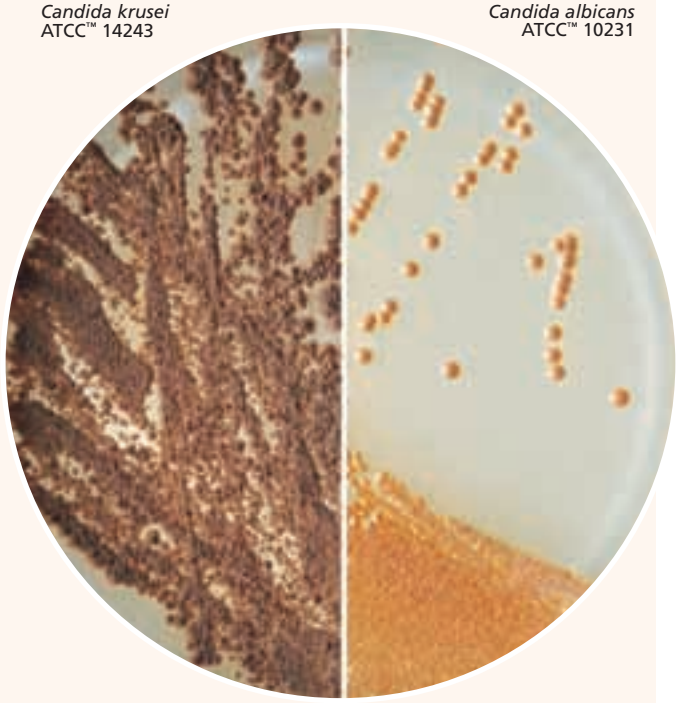
Dehydrated Appearance:	Medium fine, homogeneous, free of extraneous material.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is light to medium, cream yellow, hazy to cloudy.
Prepared Appearance:	Light to medium, cream yellow, hazy to cloudy.
Reaction of 4.5% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ BiGGY Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 25 ± 2°C for 18-24 hours (3-5 days if necessary).

ORGANISM	ATCC™	RECOVERY	COLOR OF COLONIES/MEDIUM
Candida albicans	10231	Good	Brown to black/–
Candida kefyr	8553	Good	Reddish brown/–
Candida tropicalis	1369	Good	Brown to black, metallic sheen/Brown to black
Escherichia coli	25922	Partial to complete inhibition	–/–



Formula

BBL™ BiGGY Agar

Approximate Formula* Per Liter		
Bismuth Ammonium Citrate	5.0	g
Sodium Sulfite	3.0	g
Dextrose	10.0	g
Glycine	10.0	g
Yeast Extract	1.0	g
Agar	16.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for not more than 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to approximately 45-50°C. Swirl to disperse the insoluble material and pour into plates.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for information about the processing and inoculation of specimens such as tissues, skin scrapings, hair, nail clippings, etc.[2-5] The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora. When using slants, streak the surface of the slant with a sterile inoculating loop needle using two to three isolated colonies.

Incubate plates in an inverted position (agar side up) for up to 5 days at 25 ± 2°C.

Expected Results

Within 5 days of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Slants should show evidence of growth.

Examine plates and slants for colonies showing characteristic growth patterns and morphology. The following table summarizes typical *Candida* colonial morphology.[6]

SPECIES OF CANDIDA	COLONIAL MORPHOLOGY
C. albicans	Smooth, circular or hemispherical brown-black colonies; may have slight mycelial fringe; no color diffusion into surrounding medium; no metallic sheen.
C. tropicalis	Smooth, discrete, dark brown to black colonies (may have black-colored centers); slight mycelial fringe; diffuse blackening of medium after 72 hours; metallic sheen.
C. krusei	Large, flat, wrinkled silvery brown-black colonies with brown peripheries; yellow to brown halo diffusion into medium; metallic sheen.
C. keyfr	Medium size, flat, dark reddish-brown glistening colonies; may have slight mycelial fringe; no diffusion.

References

1. Nickerson. 1953. J. Infect. Dis. 93:43.
2. Haley, Trandel and Coyle. 1980. Cumitech 11, Practical methods for culture and identification of fungi in the clinical mycology laboratory. Coord. ed., Sherris. American Society for Microbiology, Washington, D.C.
3. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
4. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ BiGGY Agar

Cat. No. 211027 Dehydrated – 500 g

United States and Canada

Cat. No. 297254 Prepared Plates – Pkg. of 20*
297255 Prepared Slants – Pkg. of 10*

Europe

Cat. No. 255002 Prepared Plates – Pkg. of 20*

Mexico

Cat. No. 252563 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Bacteroides Bile Esculin Agar (BBE)

Intended Use

Bacteroides Bile Esculin Agar (BBE) is recommended as a primary isolation medium for the selection and presumptive identification of the *B. fragilis* group.[1,2]

Summary and Explanation

Among the most frequently encountered anaerobes in human clinical infections are members of the "*Bacteroides fragilis* group". Rapid detection and identification of these organisms is important since they have been found to be more resistant to antimicrobial therapy than other anaerobes.[3] *B. fragilis* and *B. thetaiotaomicron* are the species of greatest clinical significance.[3] Other species in the group include: *B. caccae, B. distasonis, B. eggerthii, B. merdae, B. ovatus, B. stercoris, B. uniformis* and *B. vulgatus*.

Frequently these pathogens occur in a mixture of microorganisms which may overgrow the primary isolation medium. Selective media, such as CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin, have been recommended as appropriate for primary isolation.[4] However, limited evidence for the presumptive identification of the *B. fragilis* group was provided. In 1978, Livingston et al. described a primary plating medium (BBE) which was found to provide selective recovery of the *B. fragilis* group and also evidence for presumptive identification.[1]

Principles of the Procedure

Bacteroides Bile Esculin Agar is a primary plating medium for the selective isolation and presumptive identification of the *B. fragilis* group. Selective inhibition of facultative anaerobes

and most gram-negative anaerobic organisms is obtained by the presence of gentamicin and oxgall. Differentiation of the *B. fragilis* group is based on esculin hydrolysis, which produces esculetin and dextrose. The esculetin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a dark brown to black complex that appears in the medium surrounding colonies of members of the *B. fragilis* group.

Procedure

As some strains of the *B. fragilis* group may not grow well due to the selective properties of the medium, it is advisable to include a nonselective blood agar medium, such as CDC Anaerobe 5% Sheep Blood Agar. All media should be pre-reduced. Incubate immediately under anaerobic conditions (**BD GasPak**™ EZ anaerobic systems or alternative anaerobic system) for at least 48 hours at 35 ± 2°C.

Bacteroides fragilis
ATCC™ 25285

Expected Results

After 48 hours of incubation, colonies of the *B. fragilis* group should be greater than 1 mm in diameter and appear gray, circular, entire and raised. Most anaerobes other than the *B. fragilis* group are inhibited. Esculin hydrolysis is indicated by a blackening of the medium around the colonies.

Limitation of the Procedure

B. vulgatus may not hydrolyze esculin.[2,3]

References

1. Livingston, Kominos and Yee. 1978. J. Clin. Microbiol. 7:448.
2. Isenberg and Garcia (ed.), 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology. 9th ed. American Society for Microbiology, Washington, D.C.
4. Dowell, Lombard, Thompson and Armfield. 1977. Media for isolation, characterization and identification of obligately anaerobic bacteria. CDC laboratory manual. Center for Disease Control, Atlanta, Ga.

Availability

BBL™ Bacteroides Bile Esculin Agar (BBE)

BS12 CMPH2 MCM9

United States and Canada

Cat. No. 221836 Prepared Plates – Pkg. of 10*

Japan

Cat. No. 251972 Prepared Plates – Pkg. of 10*

BBL™ Bacteroides Bile Esculin Agar (BBE)// CDC Anaerobe Laked Sheep Blood Agar with KV

CMPH2 MCM9

Cat. No. 297022 Prepared **I Plate™** Dishes – Pkg. of 20*
297260 Prepared **I Plate™** Dishes – Ctn. of 100*

**Store at 2-8°C.*

Baird-Parker Agar Base • Baird-Parker Agar EY Tellurite Enrichment

Intended Use

Baird-Parker Agar Base is used with EY (Egg Yolk) Tellurite Enrichment in the preparation of Egg-Tellurite-Glycerine-Pyruvate Agar (ETGPA) for selective isolation and enumeration of coagulase-positive staphylococci from food, skin, soil, air and other materials. It may also be used for identification of staphylococci on the basis of their ability to clear egg yolk.

Summary and Explanation

A number of culture media had been utilized for the recovery of staphylococci from foods prior to the development of a new formulation by Baird-Parker in 1962.[1,2] This scientist subsequently published additional results on the efficacy of the medium for the recovery of coagulase-positive staphylococci.[3,4] In 1971, Tardio and Baer[5] and Baer[6] reported on the results of a study comparing 18 staphylococcal isolation media in which they concluded that Baird-Parker Agar should be substituted for Vogel and Johnson Agar in the official AOAC procedure for the isolation and enumeration of *Staphylococcus aureus*. In this study, it was shown that Baird-Parker Agar was less inhibitory than Vogel and Johnson Agar for selected strains of *S. aureus* and that it possesses a diagnostic aid (egg yolk reaction) not present in Vogel and Johnson Agar. The use of Baird-Parker Agar subsequently was officially adopted by AOAC International.[7]

Principles of the Procedure

Baird-Parker Agar Base contains peptone, beef extract and yeast extract as sources of nitrogenous compounds, carbon, sulfur, vitamins and trace minerals. Sodium pyruvate is incorporated

in order to stimulate the growth of *S. aureus* without destroying the selectivity. The tellurite additive is toxic to egg yolk-clearing strains other than *S. aureus* and imparts a black color to the colonies. The egg yolk additive, in addition to being an enrichment, aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). Glycine and lithium chloride have inhibitory action for organisms other than *S. aureus*.

Formulae

Difco™ Baird-Parker Agar Base

Approximate Formula* Per 950 mL

Pancreatic Digest of Casein	10.0	g
Beef Extract	5.0	g
Yeast Extract	1.0	g
Glycine	12.0	g
Sodium Pyruvate	10.0	g
Lithium Chloride	5.0	g
Agar	20.0	g

Difco™ EY Tellurite Enrichment

Egg yolk emulsion containing potassium tellurite consists of 30% egg yolk suspension with 0.15% potassium tellurite.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 63 g of the powder in 950 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C and aseptically add 50 mL of EY Tellurite Enrichment. Mix thoroughly but gently.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Food samples are macerated in suitable broth medium, diluted as desired and the dilutions spread-inoculated onto the agar surfaces, which should be dry when inoculated. Incubate plates aerobically for 24 hours at 35 ± 2°C. Consult references for detailed instructions.[7]

Expected Results

Typical colonies of *S. aureus* are black, shiny, convex and surrounded by clear zones (egg yolk reaction) of approximately 2-5 mm. Coagulase-negative staphylococci generally do not grow well; if some growth occurs, the typical clear zones are absent. The majority of other organisms are inhibited but some may grow sparsely, producing white to brown colonies with no clearing of the egg yolk.

Limitation of the Procedure

Baird-Parker Agar is selective for coagulase-positive staphylococci, but other bacteria may grow. Microscopic examination and biochemical tests will differentiate coagulase-positive staphylococci from other organisms.

User Quality Control

Identity Specifications

Difco™ Baird-Parker Agar Base

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	6.3 g/95 mL solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance (Final):	Yellow, opaque.
Reaction of 6.3 g/95 mL Solution at 25°C:	pH 6.9 ± 0.1

Difco™ EY Tellurite Enrichment

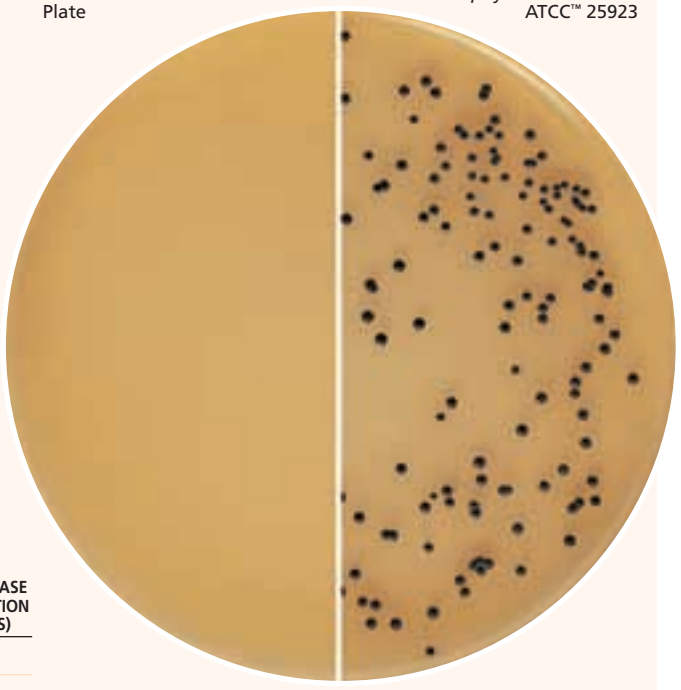
Appearance:	Canary yellow, opaque suspension with a resuspendable precipitate.

Cultural Response

Difco™ Baird-Parker Agar Base with EY Tellurite Enrichment

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-50 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	LECITHINASE PRODUCTION (HALOS)
Bacillus subtilis	6633	10^3	None to poor	Brown	–
Proteus mirabilis	25933	10^3	Good	Brown	–
Staphylococcus aureus	25923	10^2–3×10^2	Good	Black	+
Staphylococcus epidermidis	14990	10^2–3×10^2	Poor to good	Black	–



References

1. Baird-Parker. 1962. J. Appl. Bacteriol. *25*:12.
2. Baird-Parker. 1962. J. Appl. Bacteriol. *25*:441.
3. Baird-Parker. 1963. J. Gen. Microbiol. *30*:409.
4. Baird-Parker. 1965. J. Gen. Microbiol. *38*:383.
5. Tardio and Baer. 1971. J. Assoc. Off. Anal. Chem. *54*:728.
6. Baer. 1971. J. Assoc. Off. Anal. Chem. *54*:732.
7. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Baird-Parker Agar Base

AOAC BAM CCAM COMPF ISO SMD SMWW USDA

Cat. No. 276840 Dehydrated – 500 g
276810 Dehydrated – 2 kg

Difco™ EY Tellurite Enrichment

AOAC BAM CCAM COMPF ISO SMD SMWW USDA

Cat. No. 277910 Bottle – 6 × 100 mL*

BBL™ Baird-Parker Agar

AOAC BAM CCAM COMPF ISO SMD SMWW USDA

United States and Canada
Cat. No. 297214 Prepared Plates (complete) – Pkg. of 20*
297725 Prepared Plates (complete) – Ctn. of 100*

Europe
Cat. No. 255084 Prepared Plates (complete) – Pkg. of 20*

Mexico
Cat. No. 223950 Prepared Plates (complete) – Pkg. of 10*

**Store at 2-8°C.*

B

Beef Extract Powder • Bacto™ Beef Extract, Desiccated • Beef Extract

Intended Use

Beef Extract Powder, **Bacto**™ Beef Extract, Desiccated and Beef Extract (paste) are used in preparing microbiological culture media.

Summary and Explanation

Beef Extract is intended to replace aqueous infusion of meat in microbiological culture media. Beef Extract is frequently used at a concentration of 0.3 to 1.0% in culture media, although concentrations may vary depending on the nutritional requirements for the medium formulation. Beef Extract may be relied upon for biochemical studies, particularly fermentation reactions, because of its independence from fermentable substances that would interfere with the accuracy of such determinations.

Beef Extract was used in media for early studies of nonsporulating anaerobes of the intestinal tract and as a stock broth in the study of nutritional needs of streptococci. Prokofeva et al.[1] used Beef Extract for growing thermoacidophilic organisms newly isolated from hot springs in Kamchatka, Russia. Kataoka and Tokiwa[2] used Beef Extract as a nitrogen source in studies of mannose production by *Clostridium tertium* strains isolated from soil and methanogenic sludge. In addition, Beef Extract is a nutritive ingredient in many classical culture media, including Antibiotic Assay media described in *The United States Pharmacopeia*,[3] and several media recommended for standard methods applications.[4-6]

Beef Extract Powder is a meat extract dried to a powdered form.

Bacto Beef Extract, Desiccated is the dried form of Beef Extract (paste) and was developed to provide a product for ease of use in handling.

Beef Extract is a meat extract in paste form.

These products are to be used in a one for one substitution; however, variations tend to be formulation-specific and require actual performance testing.

Principles of the Procedure

Beef Extract Powder, **Bacto** Beef Extract, Desiccated and Beef Extract (paste) are derived from infusion of beef and provide an undefined source of nutrients. These Beef Extract products are not exposed to the harsh treatment used for protein hydrolysis, so they can provide some of the nutrients lost during peptone manufacture.[7] Beef Extract Powder, **Bacto** Beef Extract, Desiccated and Beef Extract (paste) are mixtures of peptides and amino acids, nucleotide fractions, organic acids, minerals and some vitamins. The function of these Beef Extract products can be described as complementing the nutritive properties of peptone by contributing minerals, phosphates, energy sources and those essential factors missing from peptone.[8]

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of Beef Extract Powder, Beef Extract, Desiccated or Beef Extract in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for specific procedures using Beef Extract Powder, **Bacto** Beef Extract, Desiccated or Beef Extract.

Expected Results

Refer to appropriate references and procedures for results.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Beef Extract, Desiccated

Dehydrated Appearance:	Medium to dark brown crystalline powder.
Solution:	0.3% solution, soluble in purified water upon warming. Solution is light to medium amber, clear.
Reaction of 0.3% Solution at 25°C:	pH 7.0 ± 0.4

Difco™ Beef Extract

Dehydrated Appearance:	Medium to dark brown paste.
Solution:	0.3% solution, soluble in purified water upon warming. Solution is light to medium amber, clear.
Reaction of 0.3% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Bacto™ Beef Extract, Desiccated

Prepare a sterile solution of 0.3% Beef Extract, Desiccated and 0.5% **Bacto** Peptone. Adjust final pH to 6.9-7.1. Inoculate and incubate tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterobacter aerogenes	13048	10^2-10^3	Good
Escherichia coli	25922	10^2-10^3	Good
Klebsiella pneumoniae	10031	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good
Shigella flexneri	12022	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good
Staphylococcus aureus	6538P	10^2-10^3	Good
Staphylococcus epidermidis	12228	10^2-10^3	Good

Difco™ Beef Extract

Prepare a sterile solution of 0.3% Beef Extract and 0.5% **Bacto** Peptone. Adjust final pH to 6.9-7.1. Inoculate and incubate tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good

Identity Specifications

BBL™ Beef Extract Powder

Dehydrated Appearance:	Fine, homogeneous, light to light medium, cream to tan.
Solution:	2.0% solution, soluble in purified water. Solution is light to light medium, yellow to tan, clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 7.0 ± 0.5

Cultural Response

BBL™ Beef Extract Powder

Prepare a sterile solution of peptone agar using 5.0 g of Beef Extract Powder, 1.25 g of sodium chloride and 3.25 g of agar in 250 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 2 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good
Streptococcus pyogenes	49117	10^2-10^3	Good

References

1. Prokofeva, Miroshnichenko, Kostrikina, Chernyh, Kuznetsov, Tourova and Bonch-Osmolovskaya. 2000. Int. J. Syst. Evol. Microbiol. *50*: Pt 6:2001.
2. Kataoka and Tokiwa. 1998. J. Appl. Microbiol. *84*:357.
3. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
4. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, DC.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
7. Cote. 1999. *In* Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York, N.Y.
8. Bridson and Brecker. 1970. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York. N.Y.

Availability

BBL™ Beef Extract Powder

AOAC COMPF SMWW

Cat. No. 212303 Dehydrated – 500 g

Bacto™ Beef Extract Desiccated

AOAC COMPF SMWW

Cat. No. 211520 Dehydrated – 500 g

Difco™ Beef Extract

AOAC COMPF SMWW

Cat. No. 212610 Dehydrated – 500 g

Beef Heart for Infusion

Intended Use

Beef Heart for Infusion is used in preparing microbiological culture media.

Summary and Explanation

Beef Heart for Infusion is prepared from fresh beef heart tissue and dried to a powdered form. Beef Heart for Infusion is processed from large quantities of raw material, retaining all the nutritive and growth-stimulating properties of the fresh tissues. One hundred grams of Beef Heart for Infusion are

the equivalent of 500 g of fresh heart tissue. Beef Heart for Infusion supplies the nutritional requirements for growth of microorganisms in Heart Infusion media.

One of the first media used for the cultivation of bacteria was a liquid medium containing an infusion of meat. Huntoon[1] used fresh beef heart and **Bacto** Peptone to prepare a "hormone" broth to retain growth-promoting substances. Highly pathogenic organisms, such as meningococci and pneumococci, could be grown on infusion medium without enrichments.[1]

Beef Heart for Infusion is a component of Heart Infusion media. Heart Infusion media are used in the mass production of microorganisms for vaccine production and are specified in standard methods for other multiple applications.[2-7]

User Quality Control

Identity Specifications

Difco™ Beef Heart for Infusion

Dehydrated Appearance:	Tan to medium brown, fine, homogeneous.
Solution:	5.0% solution, not completely soluble in purified water. Solution, after filtration, is light to medium amber, clear to slightly opalescent, may have a precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.5-7.8

Cultural Response

Difco™ Beef Heart for Infusion

Prepare a 5% solution of Beef Heart for Infusion. Infuse for one hour at 50 ± 2°C. Heat to boiling for 3-5 minutes and filter. Add 2% Proteose Peptone No. 3, 0.5% sodium chloride and 0.005% dextrose to the filtrate. Adjust pH to 7.5-7.8. Boil and filter before autoclaving. Inoculate and incubate tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Klebsiella pneumoniae	13883	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

Principles of the Procedure

Beef Heart for Infusion provides nitrogen, amino acids and vitamins in microbiological culture media.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Infusions can be prepared using 50 g of Beef Heart for Infusion per liter of purified water. For best results, infuse at 50° C for 1 hour. Heat the infusion to boiling for a few minutes to coagulate some of the proteins and filter. Add peptone and remaining ingredients of the medium to the filtrate. Adjust the pH to 7.5-7.8. Boil the medium and filter before autoclaving. Consult appropriate references for further directions on preparation of specific products.

Procedure

See appropriate references for specific procedures using Beef Heart for Infusion.[2-4]

Expected Results

Refer to appropriate references and procedures for results.

References

1. Huntoon. 1918. J. Infect. Dis. 23:168.
2. Ruoff. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
5. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, USEPA, Washington, D.C.
6. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
7. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Difco™ Beef Heart for Infusion

AOAC BAM EPA SMWW USDA

Cat. No. 213210 Dehydrated – 500 g

Bile Esculin Agar

Intended Use

Bile Esculin Agar is used to differentiate enterococci and the *Streptococcus bovis* group from other streptococci.[1,2]

Summary and Explanation

Rochaix noted the value of esculin hydrolysis in the identification of enterococci.[3] The enterococci were able to split esculin, but other streptococci could not. Meyer and Schonfeld incorporated bile into the esculin medium and showed that 61 of 62 enterococci were able to grow and split esculin, whereas the other streptococci could not.[4] Swan used an esculin medium containing 40% bile salts and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.[5]

Principles of the Procedure

Enterococci and certain streptococci hydrolyze the glycoside esculin to esculetin and dextrose. Esculetin reacts with an iron salt to form a dark brown or black complex.[6] Ferric citrate is incorporated into the medium as an indicator of esculin hydrolysis and resulting esculetin formation. Oxgall is used to inhibit gram-positive bacteria other than enterococci.

Formula

BBL™ Bile Esculin Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	5.0	g
Beef Extract	3.0	g
Oxgall	20.0	g
Ferric Citrate	0.5	g
Esculin	1.0	g
Agar	14.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 43.5 g of the powder in 1 liter of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the medium with two or three colonies and incubate overnight at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Any blackening of the plated medium indicates a positive result; if no blackening occurs, the test is negative.

For slants, if more than half of the slant is blackened within 24-48 hours, the test is positive; if less than half is blackened or no blackening occurs within 24-48 hours, the test is negative.

Limitations of the Procedure

1. Strains of *Lactococcus, Leuconostoc* and *Pediococcus* that give a positive bile-esculin reaction have been isolated from human infections.[1,2]
2. Occasional strains of viridans streptococci blacken the medium or display weakly positive reactions.[2]

User Quality Control

Identity Specifications

BBL™ Bile Esculin Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain a moderate amount of very small dark particles.
Solution:	4.35% solution, soluble in purified water upon boiling. Solution is dark, tan olive to olive green with a blue tint, trace hazy to hazy.
Prepared Appearance:	Dark, tan olive to olive green with a blue tint, trace hazy to hazy.
Reaction of 4.35% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Bile Esculin Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Enterococcus faecalis	29212	10^3-10^4	Good	Blackening
Streptococcus pyogenes	19615	10^4-10^5	Partial to complete inhibition	No blackening

References

1. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Facklam, Sahm and Teixeira. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Rochaix. 1924. Comt. Rend. Soc. Biol. *90*:771.
4. Meyer and Schonfeld. 1926. Zentralbl. Bakeriol. Parasitenk. Infectionskr. Hyg. Abt. Orig. 99:402.
5. Swan. 1954. J. Clin. Pathol. 7:160.
6. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Bile Esculin Agar

COMPF

Cat. No.	299068	Dehydrated –500 g
	221838	Prepared Plates – Pkg. of 10*
	221409	Prepared Slants – Pkg. of 10*
	221410	Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

Biosate™ Peptone

Intended Use

Biosate Peptone is used as a component in microbiological culture media or in fermentation applications.

Summary and Explanation

Biosate Peptone is a mixed hydrolysate comprised of casein and yeast extract at a ratio of 65:35. The synergistic effect of two or more types of hydrolysates is well documented and has been utilized for decades in culture media formulation. The combination of pancreatic digest of casein and yeast extract provides nutritional benefits that are not provided by the components alone. It has been reported that the combined use of these two peptones has shown improved toxin production in clostridia.[1,2] Additionally, the combination of pancreatic digest of casein and yeast extract has been used successfully as components in media which supported the hatching and culture of *Giardia* spp. from cysts and the first-time culturing of a nematode without the need of its symbiotic bacteria.[3,4]

Principles of the Procedure

Biosate Peptone provides nitrogen, amino acids and vitamins in microbiological culture media. In addition, the yeast extract component of the product provides proteins, carbohydrates and some micronutrients.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Precautions[5]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Biosate** Peptone in the formula of the medium being prepared. Add product as required.

Procedure

See appropriate references for specific procedures using **Biosate** Peptone.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Artemenko, Ivanova, Nenashev, Kuznetsova and Ochkina. 1985. Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii. *11*:37.
2. Siegel and Metzger. 1980 Appl. Environ. Microbiol. *40*:1023.
3. Ponce, Martínez and Alvarez. 1989. Archivos de Investigacion Medica. *20*:123.
4. Dorsman and Bijl. 1985. J. Parasitol. *71*:200.
5. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

User Quality Control

Identity Specifications

BBL™ Biosate™ Peptone

Dehydrated Appearance:	Yellow-tan powder, fine, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water. Solution is light to medium, yellow to tan, clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 6.3-7.5

Cultural Response

BBL™ Biosate™ Peptone

Prepare a sterile solution of peptone agar using 10 g of **Biosate** Peptone, 2.5 g of sodium chloride and 6.5 g of agar in 500 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 2-3 days (incubate *Brucella abortus* and *Streptococcus pyogenes* with 3-5% CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella abortus	11192*	10^3-10^4	Good
Escherichia coli	25922	10^4-10^5	Good
Staphylococcus aureus	6538P	10^4-10^5	Good
Streptococcus pyogenes	49117	10^3-10^4	Good

**If this strain is not available, verify performance with a known isolate.*

Availability

BBL™ Biosate™ Peptone

Cat. No.	211862	Dehydrated – 454 g
	294312	Dehydrated – 25 lb (11.3 kg)

Biotin Assay Medium

Intended Use

Biotin Assay Medium is used for determining biotin concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Biotin Assay Medium is prepared for use in the microbiological assay of biotin using *Lactobacillus plantarum* ATCC™ 8014 as the test organism.

Principles of the Procedure

Biotin Assay Medium is a biotin-free dehydrated medium containing all other nutrients and vitamins essential for the

User Quality Control

Identity Specifications

Difco™ Biotin Assay Medium

Dehydrated Appearance:	Light beige, homogeneous with a tendency to clump.
Solution:	3.75% (single strength) solution, soluble in purified water upon boiling 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Light amber, clear, may have a slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Biotin Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus plantarum* ATCC™ 8014 when prepared in single strength and supplemented with biotin. The medium should produce a standard curve when tested with a biotin reference standard at 0.0 to 1.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-20 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

cultivation of *L. plantarum* ATCC 8014. The addition of a biotin standard in specified increasing concentrations gives a growth response by this organism that can be measured titrimetrically or turbidimetrically.

Formula

Difco™ Biotin Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Acetate	20.0	g
L-Cystine	0.2	g
DL-Tryptophan	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Thiamine Hydrochloride	2.0	mg
Riboflavin	2.0	mg
Niacin	2.0	mg
Calcium Pantothenate	2.0	mg
Pyridoxine Hydrochloride	4.0	mg
p-Aminobenzoic Acid	200.0	µg
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 7.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 5 minutes.

Procedure

Stock Cultures

Stock cultures of the test organism, *L. plantarum* ATCC 8014, are prepared by stab inoculation of Lactobacilli Agar AOAC. After 16-24 hours incubation at 35-37°C, the tubes are stored in the refrigerator. Transfers are made weekly.

Inoculum

Inoculum for assay is prepared by subculturing from a stock culture of *L. plantarum* ATCC 8014 to 10 mL of single-strength Biotin Assay Medium supplemented with 0.5 ng biotin. After 16-24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are washed three times with 10 mL sterile 0.85% saline. After the third wash, the cells are resuspended in 10 mL sterile 0.85% saline and finally diluted 1:100 with sterile 0.85% saline. One drop of this suspension is used to inoculate each 10 mL assay tube.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using biotin at levels of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1 ng per assay tube (10 mL).

The concentration of biotin required for the preparation of the standard curve may be prepared by dissolving 0.1 gram of d-Biotin or equivalent in 1,000 mL of 25% alcohol solution (100 µg per mL). Dilute the stock solution by adding 2 mL to 98 mL of purified water. This solution is diluted by adding 1 mL to 999 mL purified water, giving a solution of 2 ng of biotin per mL. This solution is further diluted by adding 10 mL to 90 mL purified water, giving a final solution of 0.2 ng of biotin per mL. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 mL of this final solution. Prepare the stock solution fresh daily.

Biotin Assay Medium may be used for both turbidimetric and titrimetric analysis. Before reading, the tubes are refrigerated for 15-30 minutes to stop growth. Turbidimetric readings should be made after 16-20 hours at 35-37°C. Titrimetric determinations are made after 72 hours incubation at 35-37°C. The most effective assay range, using Biotin Assay Medium, has been found to be between 0.1 ng and 1 ng biotin.

For a complete discussion of vitamin assay methodology, refer to appropriate procedures outlined in the reference.[1]

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary by more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.

3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

Reference

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Biotin Assay Medium

Cat. No. 241910 Dehydrated – 100 g*

**Store at 2-8°C.*

Bird Seed Agar

Intended Use

Bird Seed Agar is a selective and differential medium used in the identification of *Cryptococcus neoformans*.

Summary and Explanation

Bird Seed Agar was initially described by Staib.[1] He found that *Cryptococcus neoformans* produced characteristic brown colonies when cultivated on a growth medium containing an extract prepared from the seeds of the Indian thistle plant *Guizotia abyssinica*. *C. neoformans* is the only yeast known to produce this pigmentation.[2]

Shields and Ajello later modified the original formulation by the addition of the antimicrobial agent chloramphenicol.[3] The concentration of chloramphenicol has been doubled in this medium to improve the inhibition of bacteria.

Principles of the Procedure

The seed extract contains caffeic acid, which serves as a substrate for phenol oxidase, an enzyme present in the cell wall of *C. neoformans*. The subsequent enzymatic reaction produces the brown pigment melanin, resulting in tan to brown pigmentation of the yeast colonies. *C. neoformans* is the only species known to produce this enzyme, although with occasional isolates (particularly serotype C) the production of phenol oxidase may have to be induced.[2]

The addition of the antimicrobial agent chloramphenicol improves the recovery of *Cryptococcus* from specimens containing mixed flora by suppressing bacterial growth.

Procedure

To prepare plates from agar deeps, liquefy medium in boiling water bath and pour molten medium into a sterile Petri dish; allow medium to solidify and dry before use.

Using a sterile inoculating loop or needle, pick two or three isolated colonies from the subculture medium and streak over slant or plate surface. Incubate media at 25-30°C for up to 7 days.

Expected Results

Yeast-like organisms that produce tan to brown colonies on this medium within 4-7 days may be presumptively identified as *C. neoformans*.

References

1. Staib. 1962. Z. Hyg. Infekt. Med. Mikrobiol. Immunol. *148*:466.
2. Warren and Hazen. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Shields and Ajello. 1966. Science *151*:208.

Availability

BBL™ Bird Seed Agar

Cat. No. 297875 Prepared Plates – Pkg. of 10*
297096 Prepared Pour Tubes (20 mL) – Pkg. of 10

**Store at 2-8°C.*

Bismuth Sulfite Agar

Intended Use

Bismuth Sulfite Agar is a highly selective medium used for isolating *Salmonella* spp., particularly *Salmonella* Typhi, from food and clinical specimens.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with nontyphi *Salmonella* often causes mild, self-limiting illness.[1] Typhoid fever, caused by *S.* Typhi, is characterized by fever, headache, diarrhea and abdominal pain, and can produce fatal respiratory, hepatic, splenic and/or neurological damage. These illnesses result from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. United States federal guidelines require various poultry products to be routinely monitored before distribution for human consumption but contaminated food samples often elude monitoring.

Bismuth Sulfite Agar is a modification of the Wilson and Blair[2-4] formula. Wilson[5,6] and Wilson and Blair[2-4] clearly showed the superiority of Bismuth Sulfite medium for isolation of *S.* Typhi. Cope and Kasper[7] increased their positive findings of typhoid from 1.2 to 16.8% among food handlers and from 8.4 to 17.5% among contacts with Bismuth Sulfite Agar.

Employing this medium in the routine laboratory examination of fecal and urine specimens, these same authors[8] obtained 40% more positive isolations of *S.* Typhi than were obtained on Endo medium. Gunther and Tuft,[9] employing various media in a comparative way for the isolation of typhoid from stool and urine specimens, found Bismuth Sulfite Agar most productive. On Bismuth Sulfite Agar, they obtained 38.4% more positives than on Endo Agar, 33% more positives than on Eosin Methylene Blue Agar, and 80% more positives than on the Desoxycholate media. These workers found Bismuth Sulfite Agar to be superior to Wilson's original medium. Bismuth Sulfite Agar was stable, sensitive and easier to prepare. Green and Beard,[10] using Bismuth Sulfite Agar, claimed that this medium successfully inhibited sewage organisms. The value of Bismuth Sulfite Agar as a plating medium after enrichment has been demonstrated by Hajna and Perry.[11]

Since these earlier references to the use of Bismuth Sulfite Agar, this medium has been generally accepted as routine for the detection of most *Salmonella*. The value of the medium is demonstrated by the many references to the use of Bismuth Sulfite Agar in scientific publications, laboratory manuals and texts.

For food testing, the use of Bismuth Sulfite Agar is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milks and butter.[1,12-14] The use of Bismuth Sulfite Agar is also recommended for use in testing clinical specimens.[15,16] In addition, Bismuth Sulfite Agar is valuable when investigating outbreaks of *Salmonella* spp., especially *S.* Typhi.[17-19]

Bismuth Sulfite Agar is used for the isolation of *S.* Typhi and other *Salmonella* from food, feces, urine, sewage and other infectious materials. The typhoid organism grows luxuriantly on the medium, forming characteristic black colonies, while gram-positive bacteria and members of the coliform group are inhibited. This inhibitory action of Bismuth Sulfite Agar toward gram-positive and coliform organisms permits the use of a much larger inoculum than possible with other media employed for similar purposes in the past. The use of larger inocula greatly increases the possibility of recovering the pathogens, especially when they are present in relatively small numbers. Small numbers of organisms may be encountered in the early course of the disease or in the checking of carriers and releases.

Principles of the Procedure

In Bismuth Sulfite Agar, beef extract and peptone provide nitrogen, vitamins and minerals. Dextrose is an energy source. Disodium phosphate is a buffering agent. Bismuth sulfite indicator and brilliant green are complementary in inhibiting gram-positive bacteria and members of the coliform group, while allowing *Salmonella* to grow luxuriantly. Ferrous sulfate is included for detection of H_2S production. When H_2S is present, the iron in the formula is precipitated, giving

User Quality Control

Identity Specifications

Difco™ Bismuth Sulfite Agar

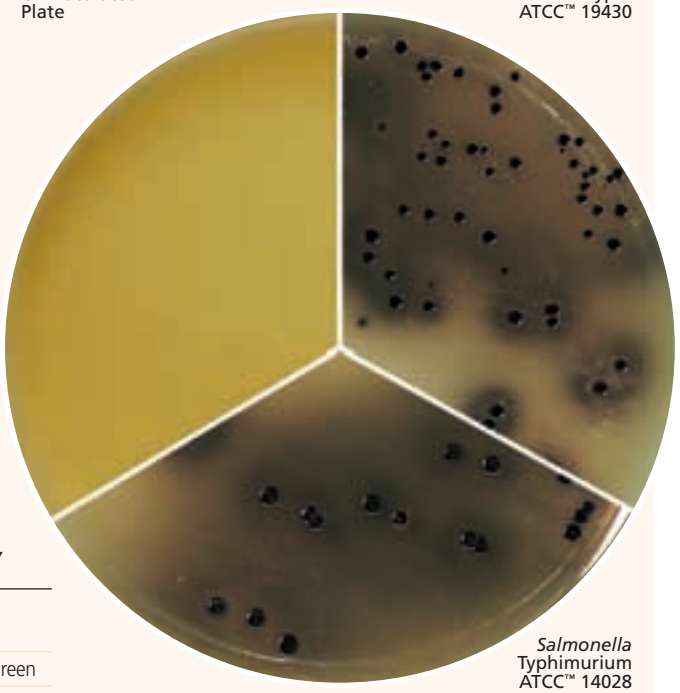
Dehydrated Appearance:	Light beige to light green, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is light green, opaque with a flocculent precipitate that can be dispersed by swirling contents of flask.
Prepared Appearance:	Light gray-green to medium green, opaque with a flocculent precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.7 ± 0.2

Cultural Response

Difco™ Bismuth Sulfite Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–
Escherichia coli	25922	10^3	Partial inhibition	Brown to green
Salmonella enterica subsp. *enterica* serotype Typhi	19430	10^2-10^3	Good	Black with sheen
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Black or greenish-gray, may or may not have sheen



positive cultures the characteristic brown to black color with metallic sheen. Agar is the solidifying agent.

Formula

Difco™ Bismuth Sulfite Agar

Approximate Formula* Per Liter		
Beef Extract	5.0	g
Peptone	10.0	g
Dextrose	5.0	g
Disodium Phosphate	4.0	g
Ferrous Sulfate	0.3	g
Bismuth Sulfite Indicator	8.0	g
Agar	20.0	g
Brilliant Green	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Evenly disperse the precipitate when dispensing. Use the medium the same day it is prepared.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For isolation of *Salmonella* spp. from food, samples are enriched and selectively enriched. Streak 10 µL of selective enrichment broth onto Bismuth Sulfite Agar. Incubate plates for 24-48 hours at 35°C. Examine plates for the presence of *Salmonella* spp. Refer to appropriate references for the complete procedure when testing food samples.[1,12-14]

For isolation of *Salmonella* spp. from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the Bismuth Sulfite Agar plate and streak for isolation. This will permit the development of discrete colonies. Incubate plates at 35°C. Examine at 24 hours and again at 48 hours for colonies resembling *Salmonella* spp.

For additional information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[15-19]

Expected Results

The typical discrete *S.* Typhi surface colony is black and surrounded by a black or brownish-black zone which may be several times the size of the colony. By reflected light, preferably daylight, this zone exhibits a distinctly characteristic metallic sheen. Plates heavily seeded with *S.* Typhi may not show this reaction except near the margin of the mass inoculation. In these heavy growth areas, this organism frequently appears as small light green colonies. This fact emphasizes the importance of inoculating plates so that some areas are sparsely populated with discrete *S.* Typhi colonies. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.

Generally, *Shigella* spp. other than *S. flexneri* and *S. sonnei* are inhibited. *S. flexneri* and *S. sonnei* strains that do grow on this medium produce brown to green, raised colonies with depressed centers and exhibit a crater-like appearance.

Escherichia coli is partially inhibited. Occasionally a strain will be encountered that will grow as small brown or greenish glistening colonies. This color is confined entirely to the colony itself and shows no metallic sheen. A few strains of *Enterobacter aerogenes* may develop on this medium, forming raised, mucoid colonies. *Enterobacter* colonies may exhibit a silvery sheen, appreciably lighter in color than that produced by *S.* Typhi. Some members of the coliform group that produce hydrogen sulfide may grow on the medium, giving colonies similar in appearance to *S.* Typhi. These coliforms may be readily differentiated because they produce gas from lactose in differential media, for example, Kligler Iron Agar or Triple Sugar Iron Agar. The hydrolysis of urea, demonstrated in Urea Broth or on Urea Agar Base, may be used to identify *Proteus* sp.

To isolate *S.* Typhi for agglutination or fermentation studies, pick characteristic black colonies from Bismuth Sulfite Agar and subculture them on MacConkey Agar. The purified colonies from MacConkey Agar may then be picked to differential tube media such as Kligler Iron Agar, Triple Sugar Iron Agar or other satisfactory differential media for partial identification. All cultures that give reactions consistent with *Salmonella* spp. on these media should be confirmed biochemically as *Salmonella* spp. before any serological testing is performed. Agglutination tests may be performed from the fresh growth on the differential tube media or from the growth on nutrient agar slants inoculated from the differential media. The growth on the differential tube media may also be used for inoculating carbohydrate media for fermentation studies.

Limitations of the Procedure

1. It is important to streak for well-isolated colonies. In heavy growth areas, *S.* Typhi appears light green and may be misinterpreted as negative growth for *S.* Typhi.[20]
2. *S.* Typhi and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. Brown zones are not produced by other members of the *Enterobacteriaceae*. However, *S. arizonae* is usually inhibited.[20]
3. Colonies on Bismuth Sulfite Agar may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium (e.g., MacConkey Agar).[20]
4. Typical *S.* Typhi colonies usually develop within 24 hours; however, all plates should be incubated for a total of 48 hours to allow growth of all typhoid strains.[20]
5. DO NOT AUTOCLAVE. Heating this medium for a period longer than necessary to just dissolve the ingredients destroys its selectivity.

References

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2. Wilson and Blair. 1926. J. Pathol. Bacteriol. *29*:310.
3. Wilson and Blair. 1927. J. Hyg. *26*:374.
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5. Wilson. 1923. J. Hyg. *21*:392.
6. Wilson. 1928. Br. Med. J. *1*:1061.
7. Cope and Kasper. 1937. J. Bacteriol. *34*:565.
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9. Gunther and Tuft. 1939. J. Lab. Clin. Med. *24*:461.
10. Green and Beard. 1938. Am. J. Public Health *28*:762.
11. Hajna and Perry. 1938. J. Lab. Clin. Med. *23*:1185.
12. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
13. Andrews, Flowers, Silliker and Bailey. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
14. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
15. Washington. 1981. Laboratory procedures in clinical microbiology. Springer-Verlag, New York, N.Y.
16. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, Mo.
17. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
18. Cintron. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
19. Grasmick. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
20. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Bismuth Sulfite Agar

AOAC BAM CCAM COMPF SMD SMWW

Cat. No. 273300 Dehydrated – 500 g

Mexico

Cat. No. 252612 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Blood Agar Base (Infusion Agar)

Intended Use

Blood Agar Base (Infusion Agar), with the addition of sterile blood, is used for the isolation, cultivation and detection of hemolytic activity of streptococci and other fastidious microorganisms.

Summary and Explanation

Infusion Agar is an all-purpose medium which has been used for many years as a base for the preparation of blood agars. In a study of viability of streptococci, Snavely and Brahier performed comparative studies of horse, rabbit and sheep blood

User Quality Control

Identity Specifications

BBL™ Blood Agar Base (Infusion Agar)

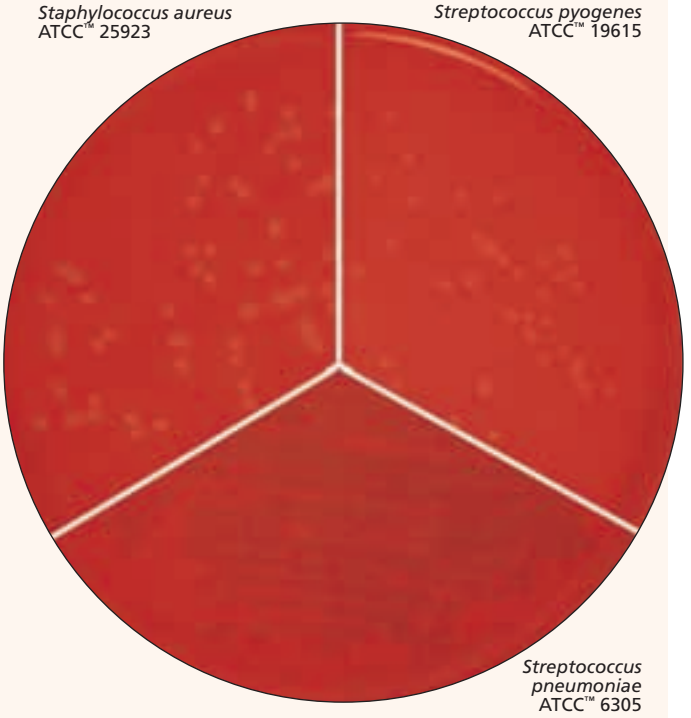
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Plain – Medium, yellow to tan, clear to slightly hazy.
	With 5% sheep blood – Cherry red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Blood Agar Base (Infusion Agar)

Prepare the medium per label directions without (plain) and with 5% defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C for 18-24 hours (incubate streptococci with 3-5% CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Candida albicans	10231	30-300	N/A	Good, no hemolysis
Listeria monocytogenes	19115	30-300	N/A	Good, beta hemolysis
Pseudomonas aeruginosa	10145	30-300	Good	N/A
Shigella flexneri	12022	30-300	Good	N/A
Staphylococcus aureus	25923	30-300	Good	Good, beta hemolysis
Streptococcus pneumoniae	6305	30-300	Good	Good, alpha hemolysis
Streptococcus pyogenes	19615	30-300	Good	Good, beta hemolysis



with Blood Agar Base, and found that sheep blood gave the clearest and most reliable colony and hemolysis characteristics at both 24 and 48 hours.[1] In the course of the investigation, about 1,300 isolations of streptococci were made with Blood Agar Base containing 5% sheep blood.

Blood Agar Base media are specified in standard methods for food testing.[2-4] Infusion Agar has been largely replaced as a blood agar base by the Tryptic/**Trypticase**™ Soy Agar formulations, which contain milk and plant peptones in place of the variable infusion component.

Principles of the Procedure

Infusion from heart muscle, casein peptone and yeast extract provide nitrogen, carbon, amino acids and vitamins in Blood Agar Base. Medium contains sodium chloride to maintain osmotic equilibrium and agar is the solidifying agent.

Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms, and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood or type of base medium used.[5]

Formula

BBL™ Blood Agar Base (Infusion Agar)

Approximate Formula* Per Liter		
Heart Muscle, Infusion from (solids)	2.0	g
Pancreatic Digest of Casein	13.0	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. For preparation of blood agar, cool the base to 45-50°C and aseptically add 5% sterile, defibrinated blood. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. After streaking, stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.[5]

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2. Incubate plates at 35 ± 2°C for 18-24 hours.

Expected Results

Colonial morphology on blood agar containing 5% sheep blood is as follows:

1. Hemolytic streptococci may appear as translucent or opaque, grayish, small (1 mm), or large matte or mucoid (2-4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.) Approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.
2. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.
3. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
4. *Listeria* may be distinguished by their rod shape in stains, and by motility at room temperature. Small zones of beta hemolysis are produced.
5. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

Limitation of the Procedure

Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta-hemolytic streptococci using other criteria.[6] The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[5]

References

1. Snavely and Brahier. 1960. Am. J. Clin. Pathol. *33*:511.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
3. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. Atlas. 1993. Handbook of microbiological media. CRC Press, Boca Raton, Fla.
5. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
6. Forbes, Sahm and Weissfeld (ed.). 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.

Availability

BBL™ Blood Agar Base (Infusion Agar)

BAM COMPF

Cat. No.	211037	Dehydrated – 500 g
	211038	Dehydrated – 5 lb (2.3 kg)

Bordet Gengou Agar Base • Bordet Gengou Blood Agar

Intended Use

Bordet Gengou Agar Base, with the addition of glycerol and sterile blood, is used in qualitative procedures for the detection and isolation of *Bordetella pertussis* from clinical specimens.

Summary and Explanation

Bordet Gengou Blood Agar is used in clinical laboratories as a method of diagnosing whooping cough. *Bordetella pertussis*, the etiologic agent of this disease, may be isolated from aspirated bronchial or nasopharyngeal secretions, perinasal swabs or, perhaps with greater difficulty due to the diversity of flora, from throat swabs.[1]

Bordet and Gengou introduced the medium in 1906 as a method of maintaining stock cultures.[2] In 1934, Kendrick and Eldering replaced the 50% human or rabbit blood recommended in the original formulation with 15% sheep blood to make the medium more practical for laboratories to produce for routine clinical procedures.[3]

Principles of the Procedure

Bordet Gengou Blood Agar contains potato infusion and glycerol to supply the nutrients necessary to support the growth of *B. pertussis*. Defibrinated animal blood supplies additional nutrients and enables the detection of hemolytic reactions, which aid in the identification of *B. pertussis*.

Formula

Difco™ Bordet Gengou Agar Base

Approximate Formula* Per Liter		
Potato, Infusion from 125 g	4.5	g
Sodium Chloride	5.5	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 30 g of the powder in 1 L of purified water containing 10 g of glycerol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add 15% sterile, defibrinated blood to the medium at 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates in an inverted position (agar side up) in a moist chamber at 35 ± 2°C for 7 days. Examine the plates daily with and without a dissecting microscope (oblique illumination) to detect the presence of *Bordetella pertussis* and spreading colonies or molds that could mask the presence of this

User Quality Control

Identity Specifications

Difco™ Bordet Gengou Agar Base

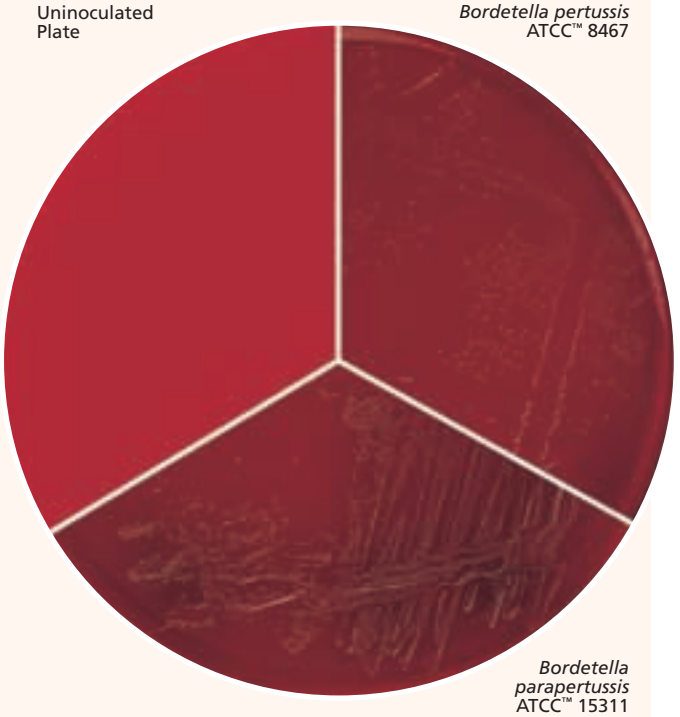
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble upon boiling in purified water containing 1% glycerol. Solution is light to medium amber, opalescent, may have a slight precipitate.
Prepared Appearance:	Plain – Light to medium amber, opalescent, may have a precipitate.
	With 15% blood – Cherry red, opaque.
Reaction of 3.0% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Bordet Gengou Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY WITH 15% RABBIT BLOOD
Bordetella bronchiseptica	4617	30-300	Good
Bordetella parapertussis	15311	30-300	Good
Bordetella pertussis	8467	30-300	Good



species. Use a sterile scalpel or needle to remove the portions of the agar that contain spreading colonies or molds. Colonies of *B. pertussis* may not be visible without the aid of a microscope for 2-4 days. Plates may be discarded as negative after incubation for 7 days.

Expected Results

Bordetella pertussis produces small, domed, glistening colonies that resemble bisected pearls. The colonies are usually surrounded by a zone of hemolysis; however, some strains of *B. pertussis* are not hemolytic. Gram stains, biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Some *Haemophilus* spp. will grow on *Bordetella* isolation media and cross-react with *B. pertussis* antisera. It may be prudent to rule out X and V factor dependence.

References

1. Loeffelholz and Sanden. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology 9th ed. American Society for Microbiology, Washington, D.C.
2. Bordet and Gengou. 1906. Ann. Inst. Pasteur *20*:731.
3. Kendrick and Eldering. 1934. Am. J. Public Health *24*:309

Availability

Difco™ Bordet Gengou Agar Base

CMPH2 MCM9

Cat. No. 248200 Dehydrated – 500 g

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

BBL™ Bordet Gengou Blood Agar

CMPH2 MCM9

Cat. No. 297876 Prepared Plates with Glycerol and 15% Sheep Blood – Pkg. of 10*

**Store at 2-8°C.*

Bovine Albumin 5%

Intended Use

Bovine Albumin 5% is used to enrich media for cultivating a large variety of microorganisms and tissue cells. Bovine albumin is also known as bovine serum albumin or BSA.[1]

Summary and Explanation

Davis and Dubos[2] recommended the use of bovine albumin at a final concentration of 0.5% in liquid media for culturing *Mycobacterium tuberculosis*. In this study, bovine albumin neutralized the toxicity of fatty acids and permitted more luxuriant growth of *M. tuberculosis*.

Ellinghausen and McCullough[3] used bovine albumin fraction V at a final concentration of 1% in liquid, semisolid and solid media for culturing leptospires. Morton et al.[4] demonstrated that 1% bovine albumin stimulated growth of *Mycoplasma* (PPLO).

User Quality Control

Identity Specifications

Difco™ Bovine Albumin 5%

Appearance:	Light amber, clear to very slightly opalescent.
Reaction of Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Bovine Albumin 5%

Prepare Dubos Broth Base per label directions, substituting Bovine Albumin 5% for Dubos Medium Albumin. Inoculate and incubate at 35 ± 2°C under CO_2 for up to 3 weeks.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Mycobacterium intracellulare	13950	10^2-10^3	Good
Mycobacterium tuberculosis H37Ra	25177	10^2-10^3	Good

Bovine Albumin can be added to normally sterile specimens, tissues and body fluids for direct inoculation onto culture media used for isolating mycobacteria. BSA is also used as an enrichment when contaminated specimens are digested.

Bovine Albumin 5%, modified with added sodium chloride and dextrose, is available as Dubos Medium Albumin.

Principles of the Procedure

Bovine Albumin 5% is a filter sterilized solution of bovine albumin fraction V. BSA is suggested as a culture media enrichment because its buffering capacity and detoxifying effect on specimen sediment.[1] Bovine Albumin 5% also increases adhesion of the specimen to solid media.[1]

Precautions[5]

1. Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet.
2. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Procedure

Sterile Specimens for the Isolation of Mycobacteria[1]

Normally sterile tissues may be ground in 0.2% BSA and inoculated directly in culture media. Concentrate body fluids before inoculation because they normally contain only a small number of mycobacteria. Centrifuge fluids at ≥ 3,000 × *g* and inoculate the sediment onto liquid or solid media. For a complete

discussion of the inoculation of sterile specimens, refer to appropriate references.

Contaminated Specimens for the Isolation of Mycobacteria[1]

A concentration of 0.2% BSA can be added to specimen sediment that has been digested and centrifuged by the N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) digestion method or by using the BBL™ MycoPrep™ Mycobacterial Specimen Digestion/Decontamination Kit. Using a separate sterile pipette for each tube, add 1-2 mL of 0.2% BSA, then resuspend the sediment with the pipette or by shaking the tube gently by hand.

Several digestion procedures exist. Consult appropriate references for a complete discussion on all digestion and decontamination methods and other testing procedures.

Expected Results

All media should be examined closely for evidence of growth. Refer to the procedure established by laboratory policy or to appropriate references on typical growth patterns and confirmation tests.

Limitation of the Procedure

Bovine Albumin 5% is not recommended for use with the BACTEC™ Blood Culture System because BSA may delay detection times.[1]

References

1. Metchock, Nolte and Wallace. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.). Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Davis and Dubos. 1945. J. Bacteriol. *55*:11.
3. Ellinghausen and McCullough. 1962. Bacteriol. Proc. *62*:54.
4. Morton, Smith, Williams and Eickenberg. 1951. J. Dent. Res. *30*:415.
5. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Difco™ Bovine Albumin 5%

Cat. No. 266810 Prepared Tubes, 20 mL – Pkg. of 12

Brain Heart CC Agar
Selective Brain Heart Infusion Agars

Intended Use

Brain Heart CC Agar is a selective medium used for the isolation of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.[1] It also serves as the base for enriched and more selective media supplemented with sheep blood and antibiotics.

Summary and Explanation

Brain Heart Infusion (BHI) Agar is recommended as a general-purpose medium for aerobic bacteriology and for the primary recovery of fungi from clinical specimens.[2,3] With 10% sheep blood, it is used to isolate systemic fungi that may grow poorly on the nonenriched medium. The presence of the antimicrobial agents, cycloheximide and/or chloramphenicol and, in modified formulations, gentamicin, penicillin and streptomycin, inhibits the growth of a wide variety of bacteria and fungi and enhances the isolation of pathogenic fungal species.

Principles of the Procedure

BHI Agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusion are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is the carbohydrate source that microorganisms utilize by fermentative action. The medium is buffered through the use of disodium phosphate. The addition of defibrinated sheep blood provides essential growth factors for the more fastidious fungal organisms. Chloramphenicol is a broad-spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria. Cycloheximide inhibits most saprophytic molds. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative and some gram-positive bacteria. Penicillin primarily inhibits gram-positive bacteria. Streptomycin inhibits gram-negative organisms.

Formula

BBL™ Brain Heart CC Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	16.0	g
Brain Heart, Infusion from (solids)	8.0	g
Peptic Digest of Animal Tissue	5.0	g
Sodium Chloride	5.0	g
Dextrose	2.0	g
Disodium Phosphate	2.5	g
Cycloheximide	0.5	g
Chloramphenicol	0.05	g
Agar	13.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 118°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

BBL™ Brain Heart CC Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to moderately hazy.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Brain Heart CC Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 25 ± 2°C under appropriate atmospheric conditions for 7 days.

ORGANISM	ATCC™	RECOVERY
Aspergillus brasiliensis (niger)	16404	Partial to complete inhibition
Candida albicans	10231	Good
Escherichia coli	25922	Partial to complete inhibition
Trichophyton mentagrophytes	9533	Good

Candida albicans
ATCC™ 10231

Procedure

Consult appropriate references for information about the processing and inoculation of specimens.[1,4]

For isolation of fungi from potentially contaminated specimens, a nonselective medium should be inoculated along with the selective medium. Incubate at 25-30°C (plates in an inverted position, agar side up, with increased humidity). For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C.

All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Expected Results

After sufficient incubation, examine cultures for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Some fungi may be inhibited by antibiotics in this medium.[5]

References

1. Reisner, Woods, Thomson, Larone, Garcia and Shimizu. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Merz and Roberts. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Brain Heart (Infusion) CC Agar

Cat No.	211057	Dehydrated – 500 g
	296261	Prepared Plates (Deep Fill) – Pkg. of 20*
	297650	Prepared Slants (A Tubes) – Pkg. of 10*
	296106	Prepared Slants (C Tubes) – Ctn. of 100*
	221834	**Mycoflask**™ Bottles – Pkg. of 10*

BBL™ Brain Heart Infusion CC Agar with Sheep Blood

Cat. No.	296178	Prepared Plates (Deep Fill) – Pkg. of 20*

BBL™ Brain Heart CC Agar with 10% Sheep Blood and Gentamicin

Cat. No.	221842	Prepared Plates (Deep Fill) – Pkg. of 10*
	296358	Prepared Slants (C Tubes) – Pkg. of 10*
	295757	Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Brain Heart Infusion Agar with 10% Sheep Blood, Gentamicin and Chloramphenicol

BS12 CMPH2 MCM9

Cat. No.	221841	Prepared Plates (Deep Fill) – Pkg. of 20*
	296343	Prepared Slants (C Tubes) – Pkg. of 10*
	295756	Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Brain Heart Infusion (Sheep) Blood Agar with Penicillin and Streptomycin

Cat. No.	296097	Prepared Plates (Deep Fill) – Pkg. of 20*
	297335	Prepared Slants (A Tubes) – Pkg. of 10*

**Store at 2-8°C.*

Brain Heart Infusion (Broth Media) Brain Heart Infusion • Brain Heart Infusion with Supplements • Brain Heart Infusion without Dextrose • Brain Heart Infusion Broth, Modified

Intended Use

Brain Heart Infusion (BHI) is a general-purpose liquid medium used in the cultivation of fastidious and nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical materials. It serves as a base for supplemented media containing 0.1% agar, Fildes enrichment or 6.5% sodium chloride. A supplemented pre-reduced formulation in tubes is especially recommended for the cultivation of anaerobes.

Summary and Explanation

Rosenow described brain-heart infusion broth prepared by adding pieces of brain tissue to meat infusion or beef extract-dextrose broth.[1] A variation of this medium appeared for many years in the National Formulary.[2] The current formulation is similar to the NF Brain Heart Infusion Broth, but the brain infusion component is composed of solids resulting from the drying of the liquid material and the heart infusion component has been replaced with a peptone of partially digested animal tissue.

BHI broth is used for the cultivation of a wide variety of microorganisms, including bacteria, yeasts and molds.

BHI broth, 0.5 mL per tube, is used for the cultivation of bacteria employed in the preparation of inocula for microdilution minimal inhibitory concentration (MIC) and identification (ID) test panels. When a large number of cells are inoculated into the small volume of broth, a bacterial culture rapidly reaches its stationary phase of growth.[3] The medium is also used in 5-mL amounts per tube for the preparation of inocula in antimicrobial susceptibility test procedures. This volume and the 8-mL tubes also can be used for general purposes.

Fildes enrichment may be incorporated for the growth of fastidious organisms. With the addition of 0.1% agar, the medium is used for the cultivation of anaerobes. The medium pre-reduced in Hungate tubes is recommended for the cultivation of anaerobic microorganisms, particularly obligate anaerobes.

The broth medium that contains 6.5% sodium chloride is used to differentiate the enterococci from nonenterococcal group D streptococci by the 6.5% salt tolerance test.[4]

Brain Heart Infusion without Dextrose is a basal medium used with carbohydrates for fermentation studies.

Brain Heart Infusion, Modified differs from other formulations by the quantities of the ingredients and the substitution of pancreatic digest of casein for pancreatic digest of gelatin.

Principles of the Procedure

BHI Broth is a nutritious, buffered culture medium that contains infusions of brain and heart tissue and peptones to supply protein and other nutrients necessary to support the growth of fastidious and nonfastidious microorganisms. In the formulation containing 6.5% sodium chloride, the salt acts as a differential and/or selective agent by interfering with membrane permeability and osmotic and electrokinetic equilibria in salt-intolerant organisms. Fildes enrichment (peptic digest of sheep blood) is incorporated into one tubed formulation for the cultivation of fastidious microorganisms, such as *Haemophilus influenzae*.[5,6] The addition of 0.1% agar aids in the cultivation of anaerobic microorganisms because its consistency yields conditions of reduced oxygen tension. The pre-reduced medium in Hungate tubes is based on Hungate methods of culturing anaerobic microorganisms outside of an anaerobic chamber.[7] The tubes provide a reduced medium in a self-contained, anaerobic tube sealed using a Hungate screw cap. The cap contains a butyl rubber septum stopper that permits inoculation and incubation without exposing the medium to air.

Formulae

Bacto™ Brain Heart Infusion

Approximate Formula* Per Liter		
Calf Brains, Infusion from 200 g	7.7	g
Beef Heart, Infusion from 250 g	9.8	g
Proteose Peptone	10.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g

BBL™ Brain Heart Infusion

Approximate Formula* Per Liter		
Brain Heart, Infusion from (solids)	6.0	g
Peptic Digest of Animal Tissue	6.0	g
Pancreatic Digest of Gelatin	14.5	g
Dextrose	3.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g

Difco™ Brain Heart Infusion without Dextrose

Approximate Formula* Per Liter		
Calf Brains, Infusion from 200 g	7.7	g
Beef Heart, Infusion from 250 g	9.8	g
Proteose Peptone	10.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Brain Heart Infusion

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2

Difco™ Brain Heart Infusion without Dextrose

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Bacto™ Brain Heart Infusion or Difco™ Brain Heart Infusion without Dextrose

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	10^2-10^3	Good
Streptococcus pneumoniae	6305	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

BBL™ Brain Heart Infusion Broth, Modified

Approximate Formula* Per Liter

Brain Heart, Infusion from (solids)	3.5	g
Peptic Digest of Animal Tissue	15.0	g
Pancreatic Digest of Casein	10.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Bacto™ Brain Heart Infusion – 37 g;
   BBL™ Brain Heart Infusion – 37 g;
   Difco™ Brain Heart Infusion without Dextrose – 35 g;
   BBL™ Brain Heart Infusion Broth, Modified – 38 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

With liquid specimens, tubed media should be inoculated with 1-2 drops of the specimen using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media.

Identity Specifications

BBL™ Brain Heart Infusion

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2

BBL™ Brain Heart Infusion Broth, Modified

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Brain Heart Infusion or BBL™ Brain Heart Infusion Broth, Modified

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 7 days (incubate *C. albicans* at 20-27°C).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY BHI	RECOVERY BHI, MODIFIED
Bacteroides fragilis	25285	≤10^4	Good	Good
Candida albicans	10231	≤10^3	Good	Good
Enterococcus faecalis	29212	≤10^3	Good	N/A
Neisseria meningitidis	13090	≤10^3	Good	Good
Streptococcus pneumoniae	6305	≤10^3	Good	Good
Streptococcus pyogenes	19615	≤10^3	Good	Good

Uninoculated Tube | *Neisseria meningitidis* ATCC™ 13090 | *Streptococcus pyogenes* ATCC™ 19615

Brain Heart Infusion

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak**™ EZ anaerobic systems or an alternative anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling with caps loosened and cooling with tightened caps to room temperature before inoculation.

Before inoculating Hungate tubes, disinfect the septum of the cap. To inoculate, insert needle of syringe containing specimen through the septum and inject the specimen into the medium. Withdraw the needle slowly to avoid introducing air into the tube.

For use in antimicrobial susceptibility testing, refer to appropriate references.[8-10]

Expected Results

Growth in the tubes is indicated by the presence of turbidity compared to an uninoculated control. If growth appears, cultures should be examined by Gram stain and subcultured onto appropriate media; e.g., a **Trypticase**™ Soy Agar with 5% Sheep Blood and/or Chocolate II Agar plate, EMB Agar or MacConkey II Agar plate. If anaerobes are suspected, subcultures should be incubated anaerobically, as in a **GasPak** EZ anaerobic system.

Enterococci will grow in the 6.5% NaCl broth within 24-48 hours. Nonenterococcal group D streptococci fail to grow in the medium after 48 hours of incubation.[3]

References

1. Rosenow. 1919. J. Dent. Res. *1*:205.
2. American Pharmaceutical Association. 1950. The national formulary, 9th ed., APA, Washington, D.C.
3. Pratt-Rippin and Pezzlo. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. Barry. 1976. The antimicrobic susceptibility test: principles and practices. Lea & Febiger, Philadelphia, Pa.
5. Fildes. 1920. Br. J. Exp. Pathol. *1*:129.
6. Fildes. 1921. Br. J. Exp. Pathol. 2.16.
7. Hungate. 1969. Methods in microbiology. Academic Press, New York, N.Y.
8. Murray, Baron, Jorgensen, Landry and Pfaller, (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
9. Clinical and Laboratory Standards Institute. 2006. Approved Standard: M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. CLSI, Wayne, Pa.
10. Clinical and Laboratory Standards Institute. 2006. Approved Standard M2-A9, Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.

Availability

Bacto™ Brain Heart Infusion

AOAC BAM CCAM CLSI CMPH2 COMPF EPA ISO MCM9 SMD SMWW USDA

Cat. No.	237400	Dehydrated – 100 g
	237500	Dehydrated – 500 g
	237200	Dehydrated – 2 kg
	237300	Dehydrated – 1 kg

BBL™ Brain Heart Infusion

AOAC BAM CCAM CLSI CMPH2 COMPF EPA ISO MCM9 SMD SMWW USDA

Cat. No.	211059	Dehydrated – 500 g
	211060	Dehydrated – 5 lb (2.3 kg)
	211061	Dehydrated – 25 lb (11.3 kg)
	221778	Prepared Tubes, 0.5 mL (K Tubes) – Ctn. of 100
	297769	Prepared Tubes, 2 mL (K Tubes) – Ctn. of 100
	221812	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10
	221813	Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100
	220837	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100
	296299	Prepared Bottles, 400 mL – 1 bottle
	297304	Prepared Tubes, Pre-reduced (with Hungate Cap) – Pkg. of 10

BBL™ Brain Heart Infusion with 6.5% Sodium Chloride

Cat. No.	221785	Prepared Tubes – Pkg. of 10

BBL™ Brain Heart Infusion with 0.1% Agar

Cat. No.	297640	Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100

BBL™ Brain Heart Infusion with Fildes Enrichment

Cat. No.	297782	Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100*
	297200	Prepared Tubes, 9 mL (K Tubes) – Pkg. of 10*

Difco™ Brain Heart Infusion without Dextrose

Cat. No.	250220	Dehydrated – 10 kg

BBL™ Brain Heart Infusion Broth, Modified

Cat. No.	299070	Dehydrated – 500 g

BBL™ Fildes Enrichment

Cat. No.	211866	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*

**Store at 2-8°C.*

Brain Heart Infusion Agars
Brain Heart Infusion Agar • Brain Heart Infusion Sheep Blood Agar • Brain Heart Infusion Agar, Modified

Intended Use

Brain Heart Infusion (BHI) Agar is a general-purpose medium suitable for the cultivation of a wide variety of organism types, including bacteria, yeasts and molds. With the addition of 5% or 10% sheep blood, it is used for the isolation and cultivation of a wide variety of fungal species, including systemic fungi,[1] from clinical and nonclinical sources.

Summary and Explanation

In the early years of bacteriology, meat infusions were utilized as the growth-supporting components in a large number of culture media. Although they were cumbersome to prepare, lacked consistency from batch to batch and were undefined as to their nutritive content, they enabled the cultivation of microorganisms in both solid and liquid media. As the state of the art in enzymology and chemistry advanced, methods were developed for the preparation of peptones that were the result of enzymatic or acid hydrolysis of animal tissues or products and vegetable substances. These peptones currently are the major nutritional additives to culture media formulations, but infusions are still utilized in specific media.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Brain Heart Infusion Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent to opalescent with a flocculent precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent to opalescent with a flocculent precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Brain Heart Infusion Agar

Prepare the medium per label directions without (plain) and with 5% defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C with 5-10% CO_2 for 18-48 hours (incubate *A. brasiliensis* aerobically at 30 ± 2°C for 18-72 hours).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Aspergillus brasiliensis (niger)	16404	10^2-3×10^2	Good	Good
Escherichia coli	25922	10^2-3×10^2	Good	Good
Staphylococcus aureus	25923	10^2-3×10^2	Good	Good
Streptococcus pneumoniae	6305	10^2-3×10^2	Good	Good
Streptococcus pyogenes	19615	10^2-3×10^2	Good	Good

BHI Agar is one formulation in which meat infusion is used, although, unlike in the earlier days, the infusion components are solids resulting from the drying of the liquid infusion material rather than the liquid components themselves. Peptones are also included as sources of nutrients.

Brain Heart Infusion Agar, Modified, the agar form of Brain Heart Infusion, Modified, differs from other formulations by the quantities of the infusion and peptone components utilized.

BHI Agar has proven to be effective in the cultivation of a wide variety of microorganisms, including many types of pathogens. BHI Agar can be used as a general medium for aerobic bacteriology and for the primary recovery of fungi from clinical specimens.[2] Brain Heart Infusion Agar with 10% Sheep Blood can be used to isolate systemic fungi that may grow poorly on the nonenriched medium. Antimicrobial agents, including chloramphenicol, gentamicin, and penicillin in combination with streptomycin, can be incorporated to improve the recovery of pathogenic fungi from specimens heavily contaminated with bacteria (see Selective Brain Heart Infusion Agars).[3]

Principles of the Procedure

BHI Agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusion are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is a carbohydrate source that

Identity Specifications

BBL™ Brain Heart Infusion Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is medium to dark, yellow to tan, trace to moderately hazy.
Prepared Appearance:	Medium to dark, yellow to tan, trace to moderately hazy.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

BBL™ Brain Heart Infusion Agar, Modified

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.3% solution, soluble in purified water upon boiling. Solution is medium to dark, yellow to tan, trace to moderately hazy.
Prepared Appearance:	Medium to dark, yellow to tan, trace to moderately hazy.
Reaction of 5.3% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Brain Heart Infusion Agar

Prepare the medium per label directions without (plain) and with 5% defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 48 hours (incubate *S. rimosus* at 23-27°C for up to 7 days if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Escherichia coli	25922	10^3-10^4	N/A	Good
Listeria monocytogenes	19115	10^3-10^4	N/A	Good
Pseudomonas aeruginosa	10145	10^3-10^4	Good	N/A
Shigella flexneri	12022	10^3-10^4	Good	N/A
Staphylococcus aureus	25923	10^3-10^4	Good	Good
Streptococcus pneumoniae	6305	10^3-10^4	Good	Good
Streptococcus pyogenes	19615	10^3-10^4	Good	Good
Streptococcus rimosus	10970	Undiluted	Good	N/A

BBL™ Brain Heart Infusion Agar, Modified

Prepare the medium per label directions without (plain) and with 5% defibrinated sheep blood (SB). Inoculate using pour plates and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Escherichia coli	25922	10^3-10^4	N/A	Good
Staphylococcus aureus	25923	10^3-10^4	Good	Good
Streptococcus pyogenes	19615	10^3-10^4	Good	Good

microorganisms utilize by fermentative action. The medium is buffered through the use of disodium phosphate.

When defibrinated sheep blood is added to the basal medium, it provides essential growth factors for the more fastidious fungal organisms.

Formulae

Difco™ Brain Heart Infusion Agar

Approximate Formula* Per Liter		
Calf Brains, Infusion from 200 g	7.7	g
Beef Heart, Infusion from 250 g	9.8	g
Proteose Peptone	10.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g
Agar	15.0	g

BBL™ Brain Heart Infusion Agar

Approximate Formula* Per Liter		
Brain Heart, Infusion from (solids)	8.0	g
Peptic Digest of Animal Tissue	5.0	g
Pancreatic Digest of Casein	16.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g
Agar	13.5	g

BBL™ Brain Heart Infusion Agar, Modified

Approximate Formula* Per Liter		
Brain Heart, Infusion from (solids)	3.5	g
Peptic Digest of Animal Tissue	15.0	g
Pancreatic Digest of Casein	10.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Brain Heart Infusion Agar – 52 g;
   BBL™ Brain Heart Infusion Agar – 52 g;
   BBL™ Brain Heart Infusion Agar, Modified – 53 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Before use, agitate gently to distribute the precipitate uniformly throughout the medium.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Prepare plated medium from tubed agar deeps by liquefying the medium in boiling water, cooling to 45-50°C and pouring into sterile Petri dishes. Additives (e.g., blood) can be used as desired.

Use standard procedures to obtain isolated colonies from specimens. Since many pathogens require carbon dioxide on primary isolation, plates of plain BHI may be incubated in an atmosphere containing approximately 5-10% CO_2. Incubate plates at 35 ± 2°C for 24-48 hours.

For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. Incubate the plates at 25-30°C in an inverted position (agar side up) with increased humidity. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

BHI Agar slants primarily are used for the cultivation and maintenance of pure cultures of microorganisms.

Expected Results

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. When culturing for fungi, examine plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Slant cultures may be used as sources of inocula for additional studies or for organism maintenance purposes.

References

1. Creitz and Puckett. 1954. Am. J. Clin. Pathol. *24*:1318.
2. Murray, Baron, Jorgensen, Landry and Pfaller, (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Reisner, Woods, Thompson, Larone, Garcia and Shimizu. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Brain Heart Infusion Agar

AOAC BAM CCAM COMPF EPA MCM9 SMD SMWW USDA

Cat. No.	241820	Dehydrated – 100 g
	241830	Dehydrated – 500 g
	241810	Dehydrated – 2 kg

BBL™ Brain Heart Infusion Agar

AOAC BAM CCAM COMPF EPA MCM9 SMD SMWW USDA

Cat. No.	211065	Dehydrated – 500 g
	212166	Dehydrated – 5 lb (2.3 kg)

United States and Canada

Cat. No.	221569	Prepared Plates (Deep Fill) – Pkg. of 20*
	221570	Prepared Plates (Deep Fill) – Ctn. of 100*
	220838	Prepared Pour Tubes (20 mL) – Pkg. of 10
	221610	Prepared Slants (K Tubes) – Pkg of 10
	297283	Prepared Slants (A Tubes) – Pkg. of 10

Europe

Cat. No.	255003	Prepared Plates – Pkg. of 20*

Japan

Cat. No.	252109	Prepared Plates (Deep Fill) – Pkg. of 20*

BBL™ Brain Heart Infusion Agar with 5% Sheep Blood

BS12 CMPH2 MCM9

Cat. No.	297199	Prepared Slants – Pkg. of 10*
	296067	Prepared Slants – Ctn. of 100*

BBL™ Brain Heart Infusion Agar with 10% Sheep Blood

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	296125	Prepared Slants – Pkg. of 10*
	221843	Prepared Plates (Deep Fill) – Pkg. of 10*

BBL™ Brain Heart Infusion Agar, Modified

Cat. No.	299069	Dehydrated – 500 g

**Store at 2-8°C.*

Bacto™ Brain Heart Infusion, Porcine

Intended Use

Bacto™ Brain Heart Infusion, Porcine is used for cultivating a wide variety of microorganisms.

Summary and Explanation

Rosenow[1] devised an excellent medium for culturing streptococci by supplementing Dextrose Broth with brain tissue. Hayden,[2] revising Rosenow's procedure by adding crushed marble to the medium, reported favorable growth of organisms from dental pathogens. Brain Heart Infusion is a modification of the media described by Rosenow[1] and Hayden.[2] Infusion from calf brains has replaced the brain tissue and disodium phosphate has replaced the calcium carbonate buffer.

Brain Heart Infusion, Porcine was developed as an alternative to the Brain Heart Infusion (BHI) formula, and replaces calf brains and beef heart with porcine brains and heart. Brain Heart Infusion, Porcine was developed for pharmaceutical and vaccine production and can replace the traditional BHI depending on organism and production application. BHI, Porcine was formulated with no bovine components to minimize Bovine Spongiform Encephalopathy (BSE) risk.

The nutritionally rich formula of BHI is used to grow a variety of microorganisms. The original Brain Heart Infusion media are specified in standard methods for multiple applications.[3-6]

Principles of the Procedure

Infusion from pork brains, infusion from pork heart and Pork Peptone No. 2 provide nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion, Porcine. Dextrose is the carbon energy source to facilitate organism growth. Sodium chloride maintains the osmotic balance of the medium. Disodium phosphate is the buffering agent.

User Quality Control

Identity Specifications

Bacto™ Brain Heart Infusion, Porcine

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Bacto™ Brain Heart Infusion, Porcine

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	10^2-10^3	Fair
Streptococcus pneumoniae	6305	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Fair

Formula

Bacto™ Brain Heart Infusion, Porcine

Approximate Formula* Per Liter		
Pork Brains, Infusion from 200 g	7.7	g
Pork Heart, Infusion from 250 g	9.8	g
Pork Peptone No. 2	10.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 37 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures using Brain Heart Infusion.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Rosenow. 1919. J. Dent. Res. *1*:205.
2. Hayden. 1923. Arch. Int. Med. *32*:828.
3. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
6. Horwitz (ed). 2007. Official methods of analysis, AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Bacto™ Brain Heart Infusion, Porcine

Cat. No.	256120	Dehydrated – 500 g
	256110	Dehydrated – 10 kg

Brain Heart Infusion with PABA
Brain Heart Infusion with PAB and Agar

Intended Use

Brain Heart Infusion (BHI) with *para*-aminobenzoic acid (PAB or PABA) is a medium used for the examination of blood from patients who have received sulfonamide therapy. The addition of agar has been found to improve growth of anaerobes.

Summary and Explanation

PAB(A) has been incorporated into the formulation for BHI to enable the detection of microorganisms in the blood of patients who are undergoing sulfonamide therapy. The addition of 0.1% agar results in a medium with improved ability to support the growth of certain microorganisms (e.g., anaerobes and microaerophiles).

Principles of the Procedure

Unsupplemented BHI broth supports the growth of a broad spectrum of microorganisms, including bacteria and fungi, due to its content of nutritive ingredients, including brain heart infusion, peptones and dextrose. Sodium chloride maintains osmotic equilibrium. PAB(A) neutralizes, by competitive inhibition, the effect of sulfonamides in the inoculum. The inclusion of agar minimizes oxygen distribution by restricting convection currents.

Formulae

Difco™ Brain Heart Infusion with PAB and Agar

Approximate Formula* Per Liter		
Calf Brains, Infusion from 200 g	7.7	g
Beef Heart, Infusion from 250 g	9.8	g
Proteose Peptone	10.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g
p-Aminobenzoic Acid	0.05	g
Agar	1.0	g

BBL™ Brain Heart Infusion with PABA

Approximate Formula* Per Liter		
Brain Heart, Infusion from (solids)	6.0	g
Peptic Digest of Animal Tissue	6.0	g
Pancreatic Digest of Gelatin	14.5	g
Dextrose	3.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g
p-Aminobenzoic Acid	0.05	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Brain Heart Infusion with PAB and Agar – 38 g;
   BBL™ Brain Heart Infusion with PABA – 37 g (for blood culture work, add 0.5 to 1.0 g of agar).
   Mix thoroughly.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Brain Heart Infusion with PAB and Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 3.8% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Brain Heart Infusion with PAB and Agar

Prepare the medium per label direction without and with 0.5 g/L of sulfadiazine. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY WITHOUT SULFADIAZINE	RECOVERY WITH SULFDIAZINE
Bacteroides fragilis	25285	30-300	Good	Good
Neisseria meningitidis	13090	30-300	Good	Good
Streptococcus pneumoniae	6305	30-300	Good	Good
Streptococcus pyogenes	19615	30-300	Good	Good

Identity Specifications

BBL™ Brain Heart Infusion with PABA

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Brain Heart Infusion with PABA

Prepare the medium per label directions without and with 0.5 g/L of sulfadiazine (do not add agar). Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 7 days (incubate *C. albicans* at 20-27°C).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY WITHOUT SULFADIAZINE	RECOVERY WITH SULFDIAZINE
Bacteroides fragilis	25285	≤ 10^4	Good	Good
Candida albicans	10231	≤ 10^4	Good	Good
Streptococcus pyogenes	19615	≤ 10^4	Good	Good

2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

With liquid specimens, tubed media should be inoculated with 1-2 drops of the specimen using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media.

Liquid tubed media for anaerobic incubation should be reduced prior to incubation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak™** EZ anaerobic system or an alternative anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling with caps loosened and cooling with tightened caps to room temperature before inoculation.

Expected Results

Examine tubes at intervals for up to 7 days for growth, which is indicated by the presence of turbidity compared to an uninoculated control.

If growth appears, cultures should be examined by Gram stain and subcultured onto appropriate media; e.g., a **Trypticase™** Soy Agar with 5% Sheep Blood and/or Chocolate II Agar plate, Eosin Methylene Blue Agar, Levine, or MacConkey II Agar plates. If anaerobes are suspected, subcultures should be incubated anaerobically, as in a **BD GasPak™** EZ anaerobic system.

Availability

Difco™ Brain Heart Infusion with PAB and Agar

Cat. No. 249910 Dehydrated – 500 g

BBL™ Brain Heart Infusion with PABA

Cat. No. 211069 Dehydrated – 500 g
220842 Prepared Tubes with 0.1% Agar, 20 mL (A Tubes) – Pkg. of 10

Brewer Anaerobic Agar

Intended Use

Brewer Anaerobic Agar is used for cultivating anaerobic and microaerophilic bacteria.

Summary and Explanation

Brewer[1] described a special Petri dish cover that allowed surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on agar with a low oxidation-reduction potential. Brewer Anaerobic Agar was originally formulated and modified for the procedure described by Brewer.[1] This medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.

Anaerobic bacteria cause a variety of infections in humans, including otitis media, oral infections, endocarditis, meningitis, wound infections following bowel surgery or trauma and bacteremia.[2-5] Anaerobic bacteria are the predominant flora colonizing the skin and mucous membranes of the body.[3] Anaerobes vary in their sensitivity to oxygen and nutritional requirements.[2] Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor.[3]

User Quality Control

Identity Specifications

Difco™ Brewer Anaerobic Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent while hot, turning red on aeration and cooling.
Prepared Appearance:	Light pink ring at outer edge, light amber in center, slightly opalescent.
Reaction of 5.8% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Brewer Anaerobic Agar

Prepare the medium per label directions. Inoculate Brewer plates with the test organisms. Replace the porous covers with Brewer covers and seal. Incubate plates at 35 ± 2°C aerobically for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	25285	10^2-10^3	Good
Clostridium beijerinckii	17795	10^2-10^3	Good
Clostridium perfringens	12924	10^2-10^3	Good

Principles of the Procedure

Peptones and yeast extract provide the nitrogen, vitamins and amino acids in Brewer Anaerobic Agar. Dextrose is the carbon source, and sodium chloride maintains osmotic equilibrium. Sodium thioglycollate and sodium formaldehyde sulfoxylate are the reducing agents. Resazurin serves as an indicator of anaerobiosis with a pink color indicating the presence of oxygen. Agar is the solidifying agent.

Formula

Difco™ Brewer Anaerobic Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	10.0	g
Yeast Extract	5.0	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
Agar	20.0	g
Sodium Thioglycollate	2.0	g
Sodium Formaldehyde Sulfoxylate	1.0	g
Resazurin	2.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 58 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Standard Petri Dishes[2]

1. Inoculate a properly obtained specimen onto the medium, and streak to obtain isolated colonies.
2. Immediately incubate anaerobically at 35 ± 2°C.
3. Examine at 24 hours if incubating plates in an anaerobic chamber. Examine at 48 hours if incubating plates in an anaerobic jar or pouch, or if using Brewer anaerobic dish cover.
4. Extended incubation may be necessary to recover some anaerobes.

Brewer Anaerobic Agar Plates

1. Dispense 50-60 mL of Brewer Anaerobic Agar into a standard Petri dish. For best results use porous tops to obtain a dry surface.
2. Inoculate the surface of the medium by streaking; avoid the edges of the plates.
3. Replace the standard Petri dish lid with a sterile Brewer anaerobic dish cover. The cover should not rest on the Petri dish bottom. The inner glass ridge should seal against the uninoculated periphery of the agar. It is essential that the sealing ring inside the cover is in contact with the medium. This seal must not be broken before the end of the incubation period. A small amount of air is caught over the surface of the medium, and the oxygen in this space reacts with the reducing agents to form an anaerobic environment.
4. Incubate aerobically as desired.

For a complete discussion on anaerobic and microaerophilic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[2-4] For the examination of anaerobic bacteria in food refer to standard methods.[6-8]

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[2]
2. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[2]
3. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure the organism is an anaerobe.[2]

References

1. Brewer. 1942. Science 95:587.
2. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
4. Murray, Baron, Jorgensen, Landry and Pfaller, (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. Smith. 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
6. Wehr and Frank (ed.). 2004. Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
7. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Brewer Anaerobic Agar

Cat. No. 227920 Dehydrated – 500 g

Brilliant Green Agar

Intended Use

Brilliant Green Agar is a highly selective medium for the isolation of *Salmonella* other than *S.* Typhi from feces and other materials.

Summary and Explanation

Brilliant Green Agar was first described by Kristensen et al. in 1925.[1] Their formulation was modified slightly by Kauffmann in 1935.[2] The medium is included in procedures for the examination of water and wastewater.[3]

Principles of the Procedure

Brilliant green dye inhibits gram-positive bacteria and a majority of gram-negative bacilli. Phenol red serves as a pH indicator and yields a yellow color as a result of acid production in the fermentation of the lactose and/or sucrose in the medium.

Formula

Difco™ Brilliant Green Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	10.0	g
Yeast Extract	3.0	g
Lactose	10.0	g
Saccharose	10.0	g
Sodium Chloride	5.0	g
Agar	20.0	g
Brilliant Green	12.5	mg
Phenol Red	0.08	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 58 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. A less selective medium and a nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Expected Results

Typical colonial morphology on Brilliant Green Agar is as follows:

Salmonella (other than *S.* Typhi and *S.* Paratyphi)	White to red, opaque colonies surrounded by red zones in the medium
S. Typhi and *S.* Paratyphi	No growth to trace growth
Shigella	No growth to trace growth
Escherichia coli and *Enterobacter/Klebsiella*	Yellow to greenish-yellow colonies surrounded by intense yellow-green zones in medium
Proteus	No growth to trace growth
Pseudomonas	Pink to red colonies
Gram-positive bacteria	No growth to trace growth

References

1. Kristensen, Lester and Jurgens. 1925. Br. J. Exp. Pathol. 6:291.
2. Kauffmann. 1935. Z. Hyg. Infektionskr. *117*:26.
3. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.

User Quality Control

Identity Specifications

Difco™ Brilliant Green Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in purified water upon boiling. Solution is brownish-green, clear to very slightly opalescent.
Prepared Appearance:	Orange-brown, very slightly to slightly opalescent.
Reaction of 5.8% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Brilliant Green Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	~10^4	Poor	Yellow-green
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	30-300	Good	Red
Salmonella enterica subsp. *enterica* serotype Typhi	19430	30-300	None to poor	Red
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Red
Staphylococcus aureus	25923	~10^4	Marked inhibition	–

Availability

Difco™ Brilliant Green Agar

EP SMWW

Cat. No. 228530 Dehydrated – 500 g

BBL™ Brilliant Green Agar

EP SMWW

Cat. No. 295963 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Brilliant Green Agar Modified

Intended Use

Brilliant Green Agar Modified is used for isolating *Salmonella* from water, sewage and foodstuffs.

Summary and Explanation

Kampelmacher[1] proposed the formula for a selective medium to isolate *Salmonella* from pig feces and minced meat. Brilliant Green Agar Modified is more selective than Desoxycholate Citrate Agar and other brilliant green media, and inhibits the growth of *Pseudomonas aeruginosa* and partially inhibits the growth of *Proteus* spp. which may resemble *Salmonella*. *Salmonella enterica* grows well on Brilliant Green Agar Modified compared to Desoxycholate Citrate Agar.[2]

Brilliant Green Agar Modified is recommended for the isolation of *Salmonella*, other than *Salmonella* Typhi, from water and associated materials[3] and meat and meat products.[4] It is recommended by the British Poultry Meat Society[5] for the examination of poultry and poultry products. The recommended procedures include using complementary selective culture media and techniques to increase the likelihood of isolating multiple serotypes of *Salmonella* from samples.[6]

User Quality Control

Identity Specifications

Difco™ Brilliant Green Agar Modified

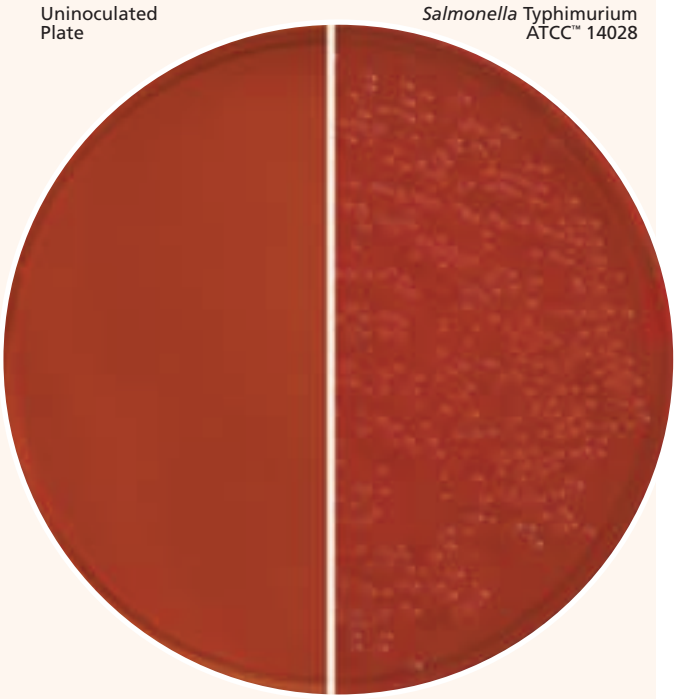
Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is orange-brown, clear to slightly opalescent.
Prepared Appearance:	Orange-brown, clear to slightly opalescent.
Reaction of 5.2% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

Difco™ Brilliant Green Agar Modified

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	10^3	Complete to partial inhibition	Green
Proteus mirabilis	25933	10^3	Complete to partial inhibition	Red
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Red



Principles of the Procedure

Brilliant Green Agar Modified contains beef extract and peptone as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Lactose and sucrose are carbohydrate sources. In the presence of phenol red, a pH indicator, lactose- and/or sucrose-nonfermenting *Salmonella* will produce red colonies. Brilliant green inhibits gram-positive organisms and many gram-negative bacteria, except *Salmonella*. Agar is the solidifying agent.

Formula

Difco™ Brilliant Green Agar Modified

Approximate Formula* Per Liter

Beef Extract	5.0	g
Peptone	10.0	g
Yeast Extract	3.0	g
Disodium Phosphate	1.0	g
Monosodium Phosphate	0.6	g
Lactose	10.0	g
Sucrose	10.0	g
Phenol Red	0.09	g
Brilliant Green	4.7	mg
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Meat and Meat Products

1. Weigh 25 g of the sample into a sterile blender jar and add 225 mL of Buffered Peptone Water. Macerate for a sufficient time to give 15,000-20,000 revolutions.
2. Aseptically transfer the contents of the blender jar to a 500 mL flask. Incubate at 37 ± 0.1°C for 16-20 hours.
3. Transfer 10 mL samples to 100 mL Muller Kauffmann Tetrathionate Broth.
4. Incubate the Muller Kauffmann Tetrathionate Broth at 42-43°C.

Sewage Polluted Natural Water

This procedure is applicable to the isolation of *Salmonella* spp. other than *S.* Typhi.

1. Inoculate 25 mL aliquots of the sample into 25 mL of double strength Buffered Peptone Water and incubate at 37°C for 18 hours.
2. Transfer 1 mL samples into 10 mL of Muller Kauffmann Tetrathionate Broth.
3. Incubate at 43°C for 48 hours.

Subculture

1. Subculture from the broth at 18-24 hours and at 48 hours onto Brilliant Green Agar Modified.
2. Examine for typical colonies of *Salmonella* after overnight incubation at 37°C.

Expected Results

Salmonella will produce red colonies.

Limitations of the Procedure

1. Organisms other than *Salmonella* spp., such as *Morganella morgani* and some *Enterobacteriaceae*, may grow on the medium.
2. Confirmatory tests, such as fermentation reactions and seroagglutination, should be carried out on all presumptive *Salmonella* spp.

References

1. Guinee and Kampelmacher. 1962. Antonie van Leeuwenhoek *28*:417.
2. Heard, Jennet and Linton. 1969. Br. Vet. J. *125*:635.
3. H. M. S. O. 1982. Methods for the isolation and identification of salmonellae (other than *Salmonella typhi*) from water and associated materials.
4. International Organisation for Standardization. 1974. Draft International Standard ISO/DIS 3565. Geneva, Switzerland.
5. British Poultry Meat Society. 1982. A manual of recommended methods for the microbiological examination of poultry and poultry products.
6. Harvey and Price. 1976. J. Hyg. Camb. *77*:333.

Availability

Difco™ Brilliant Green Agar Modified

ISO

Cat. No. 218801 Dehydrated – 500 g

Europe

Cat. No. 254490 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

B

Brilliant Green Bile Agar

Intended Use

Brilliant Green Bile Agar is used for isolating, differentiating and enumerating coliform bacteria.

Summary and Explanation

Noble and Tonney[1] described Brilliant Green Bile Agar for determining the relative density of coliform bacteria in water and sewage. The medium is particularly useful in selectively isolating *Salmonella* spp. from other coliform bacteria.

Principles of the Procedure

Brilliant Green Bile Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Lactose is a fermentable carbohydrate. Oxgall (bile) and brilliant green inhibit gram-positive bacteria and most gram-negative bacteria except coliforms. Basic fuchsin and erioglaucine are pH indicators. Monopotassium phosphate is a buffering agent. Agar is the solidifying agent.

User Quality Control

Identity Specifications

Difco™ Brilliant Green Bile Agar

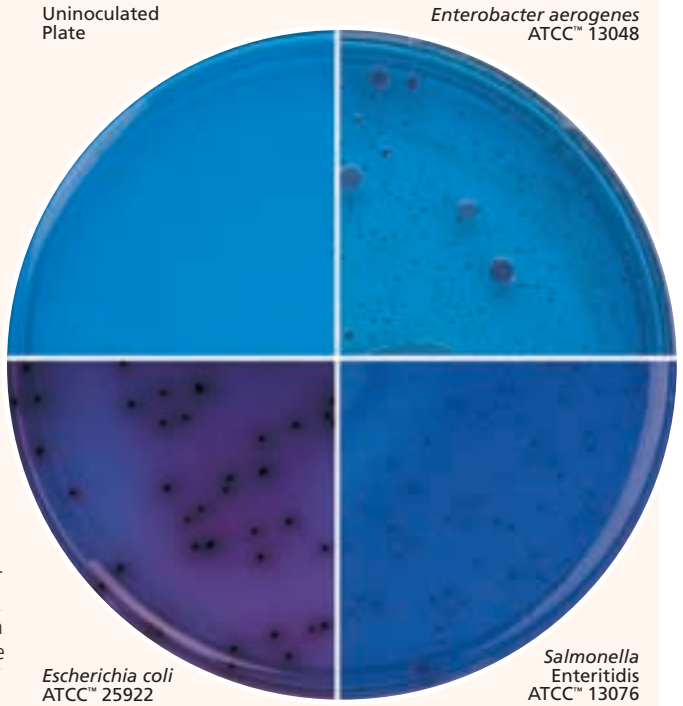
Dehydrated Appearance:	Light purple, free-flowing, homogeneous (may contain small dark particles).
Solution:	2.06% solution, soluble in purified water upon boiling. Solution is bluish-purple, slightly opalescent.
Prepared Appearance:	Blue with or without a tint of purple, slightly opalescent.
Reaction of 2.06% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Brilliant Green Bile Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterobacter aerogenes	13048	10^2-10^3	Good	Pink
Escherichia coli	25922	10^2-10^3	Good	Deep red with bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Colorless to light pink
Staphylococcus aureus	25923	10^3-2×10^3	Marked to complete inhibition	–



Differentiation of the coliforms is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of basic fuchsin, form deep red colonies with a pink halo. Bacteria that do not ferment lactose form colorless to faint pink colonies. Coliform bacteria typically ferment lactose, producing deep red colonies, while *Salmonella* spp., which do not ferment lactose, produce colorless to faint pink colonies.

Formula

Difco™ Brilliant Green Bile Agar

Approximate Formula* Per Liter	
Peptone	8.25 g
Lactose	1.9 g
Oxgall	2.95 mg
Sodium Sulfite	205.0 mg
Ferric Chloride	29.5 mg
Monopotassium Phosphate	15.3 mg
Agar	10.15 g
Erioglaucine	64.9 mg
Basic Fuchsin	77.6 mg
Brilliant Green	29.5 µg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 20.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.[2,3]

Expected Results

Refer to appropriate references and procedures for results.[2,3]

Limitation of the Procedure

The medium is sensitive to light, particularly direct sunlight, which produces a decrease in the productivity of the medium and a change in color from deep blue to purple or red. The medium should be prepared just prior to use and, when necessary to store the medium, it should be kept in the dark.

References

1. Nobel and Tonney. 1935. J. Am. Water Works Assoc. 27:108.
2. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
3. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Brilliant Green Bile Agar

COMPF

Cat. No. 214100 Dehydrated – 500 g

Brilliant Green Bile Broth 2%

Intended Use

Brilliant Green Bile Broth 2% (Brilliant Green Lactose Bile Broth) is used for the detection of coliform organisms in foods, dairy products, water and wastewater, as well as in other materials of sanitary importance.

Summary and Explanation

Brilliant Green Bile Broth 2% is formulated according to the American Public Health Association (APHA)[1] specifications for use in the confirmation of presumptive tests for coliforms.

Principles of the Procedure

Brilliant Green Bile Broth 2% contains two inhibitors of both gram-positive and selected gram-negative organisms; i.e., oxgall and brilliant green dye. Organisms, primarily coliforms, which are resistant to the action of the inhibitors and which ferment the lactose, are able to replicate in this medium. Fermentation is detected by gas production.

Formula

Difco™ Brilliant Green Bile Broth 2%

Approximate Formula* Per Liter	
Peptone	10.0 g
Oxgall	20.0 g
Lactose	10.0 g
Brilliant Green	13.3 mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes. Cool the broth as quickly as possible.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For the detailed procedures for use of this medium in confirmatory testing for coliforms, refer to the various compendia for the examination of materials of sanitary importance.[1-5]

Expected Results

Gas production within 48 ± 3 hours is considered positive evidence of fermentation by coliform bacilli. Detailed results for the enumeration of coliforms using Brilliant Green Bile Broth 2% are discussed in the various compendia of methods for microbiological examination of foods, dairy products and water and wastewater.[1-5]

User Quality Control

Identity Specifications

Difco™ Brilliant Green Bile Broth 2%

Dehydrated Appearance:	Beige to greenish-beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water. Solution is emerald green, clear.
Prepared Appearance:	Emerald green, clear.
Reaction of 4.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Brilliant Green Bile Broth 2%

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS PRODUCTION
Enterobacter aerogenes	13048	10^2-10^3	Good	+
Enterococcus faecalis	19433	10^3-2×10^3	Partial to complete inhibition	–
Escherichia coli	25922	10^2-10^3	Good	+
Staphylococcus aureus	25923	10^3-2×10^3	Marked to complete inhibition	–

Uninoculated Tube

Escherichia coli ATCC™ 25922

References

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Brilliant Green Bile Broth 2%

AOAC BAM CCAM COMPF EPA ISO SMD SMWW

Cat. No.	273000	Dehydrated – 100 g
	274000	Dehydrated – 500 g
	271000	Dehydrated – 2 kg

BBL™ Brilliant Green Bile Broth, 2%

AOAC BAM CCAM COMPF EPA ISO SMD SMWW

Cat. No.	221612	Prepared Tubes with Durham Tube – Pkg. of 10*

**Store at 2-8°C.*

m Brilliant Green Broth

Intended Use

m Brilliant Green Broth is used for recovering and differentiating *Salmonella* from primary water samples by membrane filtration.

Summary and Explanation

m Brilliant Green Broth is primarily used as a selective-differential medium for *Salmonella* species. *Salmonella* species cause many types of infections from mild, self-limiting gastroenteritis to life-threatening typhoid fever.[1] The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting less than 7 days.[1]

m Brilliant Green Broth is a modification of Kauffmann's[2] Brilliant Green Agar in which the agar has been omitted and all other ingredients are at double strength.

Kabler and Clark[3] used m Brilliant Green Broth in a membrane filtration procedure originally developed by Geldreich and Jeter.[4] In this technique, an appropriate volume of water is filtered through the membrane filter. The filter is placed on an absorbent pad saturated with m Tetrathionate Broth Base. After incubation, the membrane is transferred to another absorbent pad saturated with m Brilliant Green Broth and incubated. Following incubation, the membrane is transferred to a fresh pad saturated with urease test reagent.

Principles of the Procedure

Peptone provides the nitrogen, minerals and amino acids in m Brilliant Green Broth. Yeast extract is the vitamin source. Lactose and saccharose are the carbohydrates for bacterial growth. Sodium chloride maintains the osmotic balance of the medium and phenol red is the dye used as an indicator of carbohydrate fermentation. Brilliant green is the selective agent.

Formula

Difco™ m Brilliant Green Broth

Approximate Formula* Per Liter		
Proteose Peptone No. 3	20.0	g
Yeast Extract	6.0	g
Lactose	20.0	g
Saccharose	20.0	g
Sodium Chloride	10.0	g
Phenol Red	0.16	g
Brilliant Green	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 7.6 g of the powder in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to room temperature. Dispense 2 mL amounts onto sterile absorbent pads.
4. Use rehydrated medium within 24 hours.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Inoculate a water sample using the membrane filtration procedure.
2. Place the filter on a pad saturated with m Brilliant Green Broth.
3. Incubate at 35 ± 2°C in a humid atmosphere for 18-24 hours.
4. After incubation, examine for growth and the color of the colonies.

Expected Results

Salmonella species form pink to red colonies.

References

1. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. Kauffmann. 1935. Z. Hyg. Infektionskr. *117*:26.
3. Kabler and Clark. 1952. Am. J. Public Health 42:390.
4. Geldreich and Jeter. 1952. Abstr. Bacteriol. Proc. 52nd Gen. Meet. Soc. Am. Bacteriologists 1952.

Availability

Difco™ m Brilliant Green Broth

Cat. No. 249410 Dehydrated – 500 g

User Quality Control

Identity Specifications

Difco™ m Brilliant Green Broth

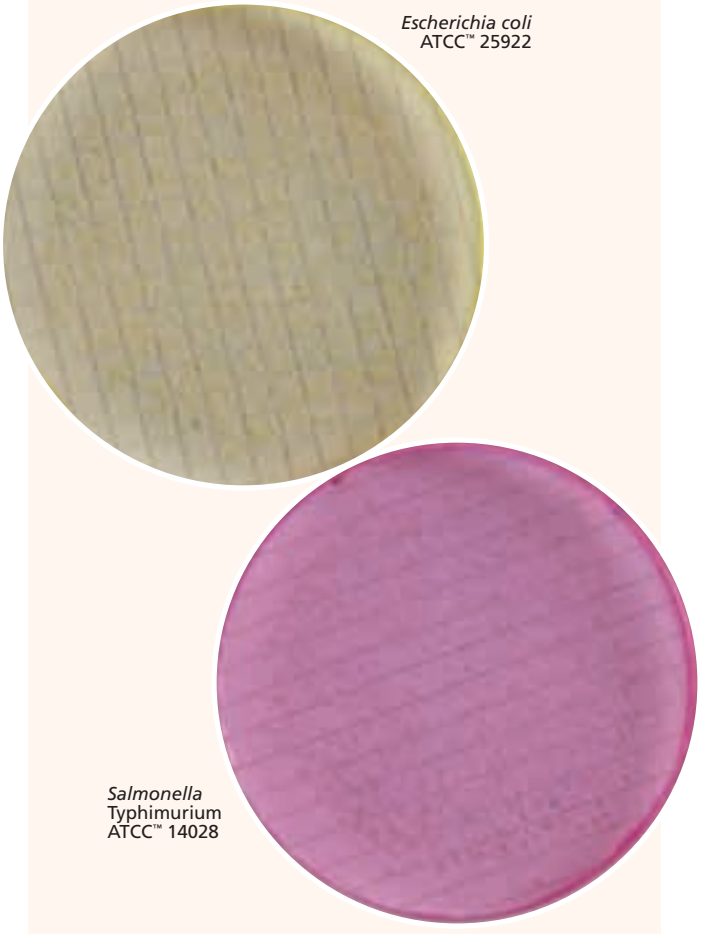
Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	7.6% solution, soluble in purified water. Solution is greenish-red, slightly opalescent.
Prepared Appearance:	Greenish-red, slightly opalescent.
Reaction of 7.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ m Brilliant Green Broth

Prepare the medium per label directions. Inoculate using the membrane filter technique and incubate at 35 ± 2°C in a humid atmosphere for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	20-80	Good	Yellow
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	20-80	Good	Pink to red
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	20-80	Good	Pink to red



Escherichia coli ATCC™ 25922

Salmonella Typhimurium ATCC™ 14028

Brucella Media
Brucella Agar • Brucella Agar with 5% Horse Blood
Brucella Broth

Intended Use

Brucella Agar is a culture medium for the cultivation of *Brucella* organisms. With the addition of 5% horse blood, the medium is used in qualitative procedures for the isolation and cultivation of nonfastidious and fastidious microorganisms from a variety of clinical and nonclinical specimens.

Brucella Broth is used for the cultivation of *Brucella* species and for the isolation and cultivation of a wide variety of fastidious and nonfastidious microorganisms.

Summary and Explanation

Brucella Agar was developed for the cultivation of *Brucella* species from diagnostic specimens, such as blood, and from foods and other potentially contaminated material. Brucella Agar with 5% Horse Blood plates are particularly useful for the cultivation of the more fastidious aerobic and anaerobic microorganisms, including streptococci, pneumococci, *Listeria*, *Neisseria meningitidis* and *Haemophilus influenzae*.

Brucella Broth may be used for the isolation and cultivation of a wide variety of microorganisms including nutritionally fastidious specimens.[1] This medium is recommended for the cultivation of *Brucella* species and was recommended as one of several media suitable for use as the liquid medium component of biphasic blood culture bottles.[1,2] It is also used to cultivate *Campylobacter* spp.[3]

Principles of the Procedure

Brucella Agar and Brucella Broth support the growth of fastidious microorganisms due to their content of peptones, dextrose and yeast extract. The peptones supply organic nitrogen. The yeast extract is a potent source of the B-complex vitamins. Dextrose is utilized as an energy source. Sodium bisulfite is a reducing agent, and sodium chloride maintains the osmotic equilibrium. Agar is the solidifying agent in Brucella Agar.

In BBL™ Brucella Agar with 5% Horse Blood plates, the horse blood supplies both the X and V factors which are growth requirements for certain organisms; e.g., *Haemophilus influenzae*.[3] Sheep and human blood are not suitable for this purpose because they contain enzymes that inactivate the nicotinamide adenine dinucleotide (NAD) which is the V factor.[4]

Defibrinated horse blood may give hemolytic reactions different than sheep blood.[5] Some streptococci (e.g., group D) give hemolytic reactions on horse blood but not on sheep blood

User Quality Control

Identity Specifications

BBL™ Brucella Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.3% solution, soluble in purified water upon boiling. Solution is light to medium, tan to yellow, clear to slightly hazy, may contain small amount of sediment.
Prepared Appearance:	Light to medium, tan to yellow, clear to slightly hazy.
Reaction of 4.3% Solution at 25°C:	pH 7.0 ± 0.2

BBL™ Brucella Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.8% solution, soluble in purified water upon heating. Solution is pale to medium, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Pale to medium, tan to yellow, clear to slightly hazy.
Reaction of 2.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

BBL™ Brucella Agar

Prepare the medium per label directions without (plain) and with 5% defibrinated horse blood (HB). Inoculate and incubate at 35 ± 2°C for 3 days with 3-5% CO_2 (incubate *S. aureus* without CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH HB
Brucella abortus	11192*	10^3-10^4	Good	Good
Brucella melitensis	4309*	10^3-10^4	Good	N/A
Brucella suis	4314*	10^3-10^4	Good	N/A
Staphylococcus aureus	25923	10^3-10^4	Good	N/A
Streptococcus pneumoniae	6305	10^3-10^4	N/A	Good
Streptococcus pyogenes	19615	10^3-10^4	N/A	Good

**Minimally one strain of* Brucella *should be used for performance testing. If these strains are not available, verify performance with a known isolate.*

BBL™ Brucella Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 7 days with 3-5% CO_2 (incubate *S. pyogenes* for 66-72 hours without CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella abortus	11192*	< 10^3	Growth
Brucella melitensis	4309*	< 10^3	Growth
Brucella suis	4314*	< 10^3	Growth
Streptococcus pyogenes	19615	< 10^3	Growth

**Minimally one strain of* Brucella *should be used for performance testing. If these strains are not available, verify performance with a known isolate.*

and may be mistakenly reported as group A. If a hemolytic reaction is obtained, the organism should be tested with a **Taxo**™ A bacitracin (0.04 unit) disc and it also should be grouped serologically or tested by the fluorescent antibody method.[6] Beta-hemolytic streptococci and *Haemophilus haemolyticus* may be differentiated by performing a Gram stain on a smear prepared from the colony.

Formulae

BBL™ Brucella Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Dextrose	1.0	g
Yeast Extract	2.0	g
Sodium Chloride	5.0	g
Sodium Bisulfite	0.1	g
Agar	15.0	g

BBL™ Brucella Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions[7]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   BBL™ Brucella Agar – 43 g;
   BBL™ Brucella Broth – 28 g.
   Mix thoroughly.
2. For the agar, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. For the broth, heat slightly, if necessary, to obtain solution.
3. Autoclave at 121°C for 15 minutes.
4. For preparation of blood plates, add 5 to 10% sterile defibrinated blood to sterile agar which has been cooled to 45-50°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Agar (without or with added blood)

Use standard procedures to obtain isolated colonies from specimens.

Since many pathogens require carbon dioxide on primary isolation, incubate plates at 35 ± 2°C for 24-72 hours in anaerobic atmosphere supplemented with carbon dioxide.

Broth

For liquid specimens, use a sterile inoculating loop to transfer a loopful to the broth medium. Swab specimens may be inserted into the broth after the inoculation of plated media.

Incubate tubes for up to 7 days at 35 ± 2°C in an aerobic atmosphere with or without supplementation with carbon dioxide.

For the preparation of biphasic blood culture bottles, aseptically add sterile Brucella Broth to a blood culture bottle containing solidified sterile Brucella Agar, with increased agar at a final concentration of 2.5%. The bottles should contain 5-10% CO_2 and be vented. Blood cultures should be incubated at 35°C for up to 30 days with subcultures prepared every 4 to 5 days.[1,2]

Expected Results

Agar (without or with added blood)

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

Broth

Growth in the tubes is indicated by the presence of turbidity compared with an uninoculated control.

If growth appears, cultures should be examined by Gram stain and subcultured onto appropriate media; e.g., **Trypticase**™ Soy Agar with 5% Sheep Blood and/or Brucella Agar and Chocolate II Agar, Eosin Methylene Blue Agar, Levine or MacConkey II Agar.

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Moyer, Holcomb and Hausler. 1991. *In* Balows, Hausler, Herrmann, Isenberg, and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
3. Chapin and Murray. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.). Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Krumweide and Kuttner. 1938. J. Exp. Med. *67*:429.
5. Vera and Power. 1980. *In* Lennette, Balows, Hausler and Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
6. Vera. 1971. Health Lab. Sci. *8*:176.
7. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Brucella Agar

CCAM ISO USDA

Cat. No.	211086	Dehydrated – 500 g
	221547	Prepared Plates with 5% Horse Blood – Pkg. of 20*
	221548	Prepared Plates with 5% Horse Blood – Ctn. of 100*

BBL™ Brucella Broth

CCAM ISO USDA

Cat. No.	211088	Dehydrated – 500 g
	296185	Prepared Tubes (K Tubes), 5 mL – Ctn. of 100

**Store at 2-8°C.*

Brucella Media for Anaerobes
Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K_1 • Brucella Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV)

Intended Use

Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K_1 is used for the isolation and cultivation of fastidious, obligately anaerobic microorganisms.

Brucella Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV) is used for the selective isolation of fastidious and slow growing, obligately anaerobic bacteria from the same specimen.

Summary and Explanation

The isolation of obligately anaerobic bacteria from clinical and nonclinical materials requires the use of selective, nonselective and enrichment media.[1] Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K_1 is an enriched, nonselective medium for the isolation and cultivation of a wide variety of obligately anaerobic microorganisms. Nonselective media are used to isolate organisms present in low numbers and to provide an indication of the numbers and types of organisms present in the specimen or sample.

Kanamycin and vancomycin are included in Brucella Laked Blood KV Agar for use in selective isolation of gram-negative anaerobes, especially *Bacteroides*. The combination of kanamycin and vancomycin for this purpose was first described by Finegold et al.[2] Vancomycin, however, may inhibit *Porphyromonas asaccharolytica*.[3]

Principles of the Procedure

Brucella Agar supports the growth of fastidious microorganisms due to its content of peptones, dextrose and yeast extract. The sheep blood, hemin and vitamin K_1, provide essential nutrients for certain obligate anaerobes.[4]

The addition of the antimicrobial agents, kanamycin and vancomycin, renders Brucella Laked Blood KV Agar selective for gram-negative microorganisms. The kanamycin inhibits protein synthesis in susceptible organisms, whereas the vancomycin inhibits gram-positive bacteria by interfering with cell wall synthesis.[5] The laked blood improves pigmentation of the *Prevotella melaniogenica - P. asaccharolytica* group.

Procedure

These media should be reduced immediately prior to inoculation by placing under anaerobic conditions for 18-24 hours.[6] An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak**™ EZ anaerobic systems or an alternative system.[7]

Streak the specimen as soon as possible after it is received in the laboratory. Minimize exposure to air. With liquid specimens, media should be inoculated with one drop of the specimen. Tissue specimens should be minced and then ground in sterile broth such as **BBL** Enriched Thioglycollate Medium before inoculation. Inoculation is then performed as for liquid specimens. Swab specimens may be rolled onto the first quadrant of plated media and then used to inoculate liquid media. Alternately, the swab may be "scrubbed" in a small volume of reduced broth and the broth used to inoculate media as performed with liquid specimens.

An enrichment broth such as **BBL** Enriched Thioglycollate Medium should be inoculated at the same time as the primary isolation plates.

Incubate immediately under anaerobic conditions or place in a holding jar flushed with oxygen-free gas(es) until sufficient plates are accumulated (but no longer than 3 hours).[8] Incubation should be at 35 ± 2°C for at least 48 hours and up to 7 days. Regardless of anaerobic system used, it is important to include an indicator of anaerobiosis such as a **GasPak** anaerobic indicator.

Expected Results

Examine colonies using a dissecting microscope and with a long-wave UV lamp to detect fluorescence. Colonies of the pigmenting *Bacteroides* group should fluoresce orange to brick-red under long-wave UV light. Fluorescence is visible before pigmentation.

In order to determine the relationship to oxygen of each colony type present on anaerobic solid media, follow established procedures.[9] Those colony types that prove to contain obligate anaerobes can be further studied using appropriate identification methods. Consult appropriate texts for additional information.[10,11]

References

1. Dowell. 1975. Am. J. Med. Technol. *41*:402.
2. Finegold, Miller and Posnick. 1965. Ernahrungsforschung *10*:517.
3. Van Winkelhoff and de Graaff. 1983. J. Clin. Microbiol. *18*:1282.
4. Gibbons and MacDonald. 1960. J. Bacteriol. *80*:164.
5. Estevez. 1984. Lab. Med. *15*:258.
6. Dowell and Hawkins. 1987. Laboratory methods in anaerobic bacteriology. CDC laboratory manual. HHS Publication No. (CDC) 87-8272. Centers for Disease Control, Atlanta, Ga.
7. Seip and Evans. 1980. J. Clin. Microbiol. *11*:226.
8. Martin. 1971. Appl. Microbiol. *22*:1168.
9. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
10. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.
11. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Brucella Agar with 5% Sheep Blood, Hemin and Vitamin K_1

BS12 CMPH2 MCM9

United States and Canada

Cat. No. 297848 Prepared Plates – Pkg. of 20*
297716 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 255509 Prepared Plates – Ctn. of 20*

BBL™ Brucella Laked Sheep Blood Agar with Kanamycin and Vancomycin

BS12 MCM9

Cat. No. 297840 Prepared Plates – Pkg. of 20*

BBL™ Brucella 5% Sheep Blood Agar with Hemin and Vitamin K_1//Brucella Laked Sheep Blood Agar with Kanamycin and Vancomycin

Cat. No. 297849 Prepared **I Plate™** Dishes – Pkg. of 20*

**Store at 2-8°C.*

Brucella Broth with 20% Glycerol

Intended Use

This medium is used in the long-term frozen maintenance of bacterial stock cultures.

Summary and Explanation

Brucella Broth is a nutritious medium that, when supplemented with glycerol, may be used as a maintenance medium for the preservation of bacterial cultures.[1,2]

Principles of the Procedure

Enzymatic digest of protein substrates act as protective colloids.

Glycerol is a cryoprotective agent that provides intracellular and extracellular protection against freezing.[2]

Procedure

Using a sterile swab or inoculating loop, remove fresh growth from the plated or slanted medium and suspend in the broth maintenance medium to achieve the desired concentration of viable cells. Freeze suspension immediately at –20°C or below. Consult texts for detailed information about preparing stock cultures of specific organisms.[2-4]

Expected Results

Bacterial stock cultures frozen and stored at –20°C or below will remain viable for several months, and some may remain viable for years.

Limitations of the Procedure

The appropriate procedure, storage temperature, length of storage, etc., may vary for specific organisms.

References

1. MacFaddin. 1985. Media for isolation-cultivation-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Gherna. 1994. *In* Gerhardt, Murray, Wood and Krieg (ed.), Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
3. Norris and Ribbons (ed.). 1970. Methods in microbiology, vol. 3A. Academic Press, Inc., New York, N.Y.
4. Kirsop and Snell (ed.). 1984. Maintenance of microorganisms. Academic Press, Inc., New York, N.Y.

Availability

BBL™ Brucella Broth with 20% Glycerol

Cat. No. 297466 Prepared Tubes – Pkg. of 10

Buffered Listeria Enrichment Broth Base

(See Listeria Enrichment Broth)

Buffered Peptone Water
Buffered Peptone Casein Water

Intended Use

Buffered Peptone Water and Buffered Peptone Casein Water are used for preenriching injured *Salmonella* species from food specimens to increase recovery.

Summary and Explanation

Edel and Kampelmacher[1] noted that food preservation techniques involving heat, desiccation, preservatives, high osmotic pressure or pH changes cause sublethal injury to salmonellae. Preenrichment in a nonselective medium allows for repair of cell damage and facilitates the recovery of salmonellae. Lactose Broth is frequently used for this purpose but it may be detrimental to recovering salmonellae.[2] Buffered Peptone Water maintains a high pH over the preenrichment period and results in repair of injured cells that may be sensitive to low pH.[3] This is particularly important for vegetable specimens which have a low buffering capacity. Buffered Peptone Water can be used for testing dry poultry feed.[4] Test methods have been published for a variety of food samples.[5,6] Casein peptone in Buffered Peptone Casein Water conforms with ISO 6579:2002.[7]

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Buffered Peptone Water or Difco™ Buffered Peptone Casein Water

Dehydrated Appearance:	Cream-white to light biege, free flowing, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water. Solution is light amber, clear to slightly hazy.
Prepared Appearance:	Light amber, clear.
Reaction of 2.0% Solution at 25°C:	pH 7.2 ± 0.2 (Peptone Water) pH 7.0 ± 0.2 (Casein Water)

Cultural Response

Difco™ Buffered Peptone Water or Difco™ Buffered Peptone Casein Water

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PEPTONE WATER	RECOVERY CASEIN WATER
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10-100	Good	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10-100	Good	Good
Salmonella enterica subsp. *enterica* serotype Typhi	19430	10-100	Good	Good
Salmonella panama ALM 41		10-100	N/A	Good

Identity Specifications

BBL™ Buffered Peptone Water

Dehydrated Appearance:	Cream-white to light tan, free flowing, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water. Solution is light yellow to tan or amber, clear to slightly hazy.
Prepared Appearance:	Light yellow to tan or amber, clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ Buffered Peptone Water

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10-10^3	Good
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhi	19430	10-10^3	Good

Principles of the Procedure

These preenrichment media contain peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance. Phosphates buffer the media.

Formulae

Difco™ or BBL™ Buffered Peptone Water

Approximate Formula* Per Liter

Peptone	10.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	3.5	g
Monopotassium Phosphate	1.5	g

Difco™ Buffered Peptone Casein Water

Approximate Formula* Per Liter

Enzymatic Digest of Casein	10.0	g
Sodium Chloride	5.0	g
Disodium Hydrogen Phosphate (anhydrous)†	3.5	g
Potassium Dihydrogen Phosphate	1.5	g

† Anhydrous Disodium Hydrogen Phosphate (3.5 g) is equivalent to 9.0 g of Disodium Phosphate Dodecahydrate.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve the powder in 1 L of purified water:
   Difco™ or BBL™ Buffered Peptone Water – 20 g;
   Difco™ Buffered Peptone Casein Water – 20 g.
   Mix thoroughly.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Refer to appropriate references for details on test methods using these media.[5-7] Inoculate tubes with the test sample. Incubate tubes at 35 ± 2°C for 18-24 hours in an aerobic atmosphere, or as instructed in the appropriate reference.[5-7]

Expected Results

Growth is indicated by turbidity.

Limitation of the Procedure

The types and numbers of competing flora in the test sample can affect recovery and may overgrow salmonellae.

References

1. Edel and Kampelmacher. 1973. Bull. W.H.O. *48*:167.
2. Angelotti. 1963. Microbiological quality of foods. Academic Press, New York, N.Y.
3. Sadovski. 1977. J. Food Technol. *12*:85.
4. Juven, Cox, Bailey, Thomson, Charles and Schutze. 1984. J. Food Prot. *47*:299.
5. Andrews, Flowers, Silliker and Bailey. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Rose. 2001. Isolation and identification of *Salmonella* from meat, poultry and egg products. *In* Microbiology laboratory guidebook, 3rd ed., Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C.
7. International Organization for Standards (ISO). Microbiology of food and animal feeding stuffs – horizontal method for the detection of *Salmonella* spp., 4th ed., ISO 6579:2002.

Availability

Difco™ Buffered Peptone Water

BAM CCAM ISO USDA

Cat. No.	218105	Dehydrated – 500 g
	218103	Dehydrated – 2 kg
	218104	Dehydrated – 10 kg

BBL™ Buffered Peptone Water

BAM CCAM ISO USDA

Cat. No.	212367	Dehydrated – 500 g
	212345	Dehydrated – 5 lb (2.3 kg)

Difco™ Buffered Peptone Casein Water

ISO

Cat. No.	214939	Dehydrated – 500 g
	214938	Dehydrated – 10 kg

Buffered Sodium Chloride-Peptone Solution pH 7.0

Intended Use

Buffered Sodium Chloride-Peptone Solution pH 7.0 is used for dissolving, suspending and diluting test samples.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Buffered Sodium Chloride-Peptone Solution pH 7.0 is used to make suspensions of organisms for testing growth promoting and inhibitory properties of media when examining non-sterile pharmaceutical products for specified microorganisms.[1] This fluid provides osmotic stability, a stable pH value and maintains the viability of microorganisms during preparation of samples. Surface-active ingredients or inactivators of antimicrobial agents such as (but not limited to) polysorbate 80 may be added to Buffered Sodium Chloride-Peptone Solution pH 7.0.

User Quality Control

Identity Specifications

Buffered Sodium Chloride-Peptone Solution pH 7.0 (prepared)

Appearance:	Colorless, clear.
Reaction at 25°C:	pH 7.0 ± 0.2

Survival Test

Buffered Sodium Chloride-Peptone Solution pH 7.0 (prepared)

Perform a 2-hour survival test. Grow test strains overnight in TSB and inoculate cultures into test solution. At time zero (directly after inoculation) and after 2 hours incubation at room temperature, subculture to Tryptic/**Trypticase**™ Soy Agar and incubate at 30-35°C for 18-24 hours (subculture (*) cultures to Sabouraud Dextrose Agar and incubate at 20-25°C for 2-3 days). Determine colony counts at time zero and after 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AT 2 HOURS
Aspergillus brasiliensis (*niger*)*	16404	10^2-10^3	≥ 100% of time zero counts
Bacillus subtilis	6633	10^2-10^3	≥ 100% of time zero counts
*Candida albicans**	10231	10^2-10^3	≥ 100% of time zero counts
Escherichia coli	8739	10^2-10^3	≥ 100% of time zero counts
Pseudomonas aeruginosa	9027	10^2-10^3	≥ 100% of time zero counts
Salmonella Abony DSM 4224		10^2-10^3	≥ 100% of time zero counts
Staphylococcus aureus	6538	10^2-10^3	≥ 100% of time zero counts

Principles of the Procedure

Phosphates are the buffering agents in the solution. Sodium chloride provides osmotic stability. A low peptone content provides basic nutrients such as amino acids to maintain organism viability.

Formula

Buffered Sodium Chloride-Peptone Solution pH 7.0

Approximate Formula* Per Liter		
Proteose Peptone No.3	1.0	g
Potassium Dihydrogen Phosphate	3.6	g
Disodium Hydrogen Phosphate (Dihydrate)	7.2	g
Sodium Chloride	4.3	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Procedure

Refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

For details on test methods for the examination of nonsterile pharmaceutical products using Buffered Sodium Chloride-Peptone Solution pH 7.0, refer to *USP* General Chapter <62>.

Limitation of the Procedure

Buffered Sodium Chloride-Peptone Solution pH 7.0 is not a culture medium. The minimal nutrient content does not allow significant growth of more fastidious microorganisms. Instead, transfer aliquots of the processed solutions or the inoculated filter membranes to suitable culture media.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.

Availability

BD™ Buffered Sodium Chloride-Peptone Solution pH 7.0

CCAM EP JP USP

Cat No.	257086	Prepared Bottles, 100 mL (stopper with ring) – Ctn. of 25†
	257087	Prepared Bottles, 500 mL (stopper with ring) – Pkg. of 10†

†QC testing performed according to USP/EP/JP performance specifications.

Bushnell-Haas Broth

Intended Use

Bushnell-Haas Broth is used for studying microbial utilization of hydrocarbons.

Summary and Explanation

Bushnell-Haas Broth (Bushnell-Haas marine salts broth), prepared according to the formula described by Bushnell and Haas[1], is used to evaluate the ability of microorganisms to decompose hydrocarbons. It is formulated without a carbon source which allows for the addition of alternative hydrocarbons such as kerosene, light and heavy mineral oils, paraffin wax and gasoline.

Bushnell-Haas Broth was recommended for the microbiological examination of fuels by the Society for Industrial Microbiology (SIM) Committee on Microbiological Deterioration of Fuels.[2] The medium was used to enumerate total heterotrophs and hydrocarbon degradation by microorganisms during bioremediation of Prince William Sound following the Exxon *Valdez* oil spill.[3,4]

Principles of the Procedure

Magnesium sulfate, calcium chloride and ferric chloride provide trace elements necessary for bacterial growth. Potassium nitrate is a nitrogen source, while monopotassium phosphate and diammonium hydrogen phosphate provide buffering capability.

User Quality Control

Identity Specifications

Difco™ Bushnell-Haas Broth

Dehydrated Appearance:	Beige with pink tint, free-flowing, homogeneous (may contain small dark particles).
Solution:	0.327% solution, partially soluble in purified water, white precipitate remains. Solution, after autoclaving, is colorless to very light amber, clear supernatant over yellow-orange precipitate.
Prepared Appearance:	Colorless to very light amber, clear supernatant over yellow-orange precipitate.
Reaction of 0.327% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Bushnell-Haas Broth

Prepare the medium per label directions. Inoculate in duplicate with the test organisms. Add sterile mineral oil (the hydrocarbon source) to one set. Incubate at 25-30°C for up to 1 week.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY WITHOUT HYDROCARBON	RECOVERY WITH HYDROCARBON
Pseudomonas aeruginosa	9027	10^2-10^3	None to poor	Good
Pseudomonas aeruginosa	10145	10^2-10^3	None to poor	Good
Pseudomonas aeruginosa	14207	10^2-10^3	None to poor	Good
Pseudomonas aeruginosa	27853	10^2-10^3	None to poor	Good

Formula

Difco™ Bushnell-Haas Broth

Approximate Formula* Per Liter		
Magnesium Sulfate	0.2	g
Calcium Chloride	0.02	g
Monopotassium Phosphate	1.0	g
Diammonium Hydrogen Phosphate	1.0	g
Potassium Nitrate	1.0	g
Ferric Chloride	0.05	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 3.27 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: A precipitate, white prior to autoclaving becoming yellow to orange after autoclaving, is normal.

Procedure

1. Inoculate the collected sample directly into the broth.
2. Overlay the broth with a sterile hydrocarbon source.
3. Incubate aerobically at 25-30°C.
4. Examine tubes daily for growth for up to one week.

Expected Results

Organisms capable of degrading hydrocarbons should show growth in the Bushnell-Haas Broth supplemented with a hydrocarbon source.

References

1. Bushnell and Haas. 1941. J. Bacteriol. *41*:653.
2. Allred, DeGray, Edwards, Hedrick, Klemme, Rogers, Wulf and Hodge. 1963. Proposed procedures for microbiological examination of fuels. SIM Special Publications, No. 1. Merck, Sharp & Dohme Research Laboratories, Rahway, N.J.
3. Bragg, Roffall and McMillen. 1990. Column flow studies of bioremediation in Prince William Sound. Exxon Production Research Co., Houston, Tex.
4. Brown and Braddock. 1990. Appl. Environ. Microbiol. *56*:3895.

Availability

Difco™ Bushnell-Haas Broth

Cat. No. 257820 Dehydrated – 500 g

CDC Anaerobe 5% Sheep Blood Agar
CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin (KV) • CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol (PEA)
CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV)

Intended Use

CDC Anaerobe 5% Sheep Blood Agar is used for the isolation and cultivation of fastidious and slow growing, obligately anaerobic bacteria from a variety of clinical and nonclinical materials. It also supports good growth of most aerobic, facultatively anaerobic and microaerophilic bacteria if incubated appropriately.

CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin (KV), CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol (PEA) and CDC Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin (KV) are used for the selective isolation of fastidious and slow-growing, obligately anaerobic bacteria from a variety of clinical and nonclinical materials.

Summary and Explanation

The isolation of obligately anaerobic bacteria from clinical and nonclinical materials requires the use of selective, nonselective and enrichment media.[1] The choice of media to be employed is based upon the type of material and the results of direct microscopic observation. Nonselective media are used to isolate organisms present in low numbers and to provide an indication of the numbers and types of organisms present in the specimen or sample. Selective media are employed to facilitate recovery of the desired organisms present in mixed populations.

CDC Anaerobe 5% Sheep Blood Agar was formulated by Dowell et. al. of the Center for Disease Control (currently named the Centers for Disease Control and Prevention CDC) as an enriched, nonselective medium for the isolation and cultivation of a wide variety of obligately anaerobic microorganisms, particularly those found in clinical materials.[2-5] The medium employs **BBL**™ **Trypticase**™ Soy Agar supplemented with additional agar, yeast extract, vitamin K_1, hemin, cystine and 5% sheep blood. Improved growth of *Prevotella melaninogenica, Fusobacterium necrophorum, Clostridium haemolyticum*, as well as certain strains of *Actinomyces israelii* and *Bacteroides thetaiotaomicron*, has been demonstrated on this medium.[3] Furthermore, less smooth to rough colonial variation has been reported on this medium than on Schaedler Blood Agar.[6]

CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin was formulated as an enriched, selective medium for the isolation of obligately anaerobic, gram-negative bacilli from clinical materials.[2] The medium employs **Trypticase** Soy Agar supplemented with additional agar, yeast extract, vitamin K_1, hemin, cystine, 5% sheep blood, kanamycin and vancomycin. The combination of kanamycin and vancomycin for use in selective isolation of gram-negative anaerobes was first described by Finegold et. al.[7] Vancomycin, however, may inhibit *Porphyromonas asaccharolytica*.[8]

CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol was formulated as an enriched selective medium for the isolation and cultivation of obligately anaerobic bacteria from clinical materials containing rapidly growing, facultatively anaerobic bacteria such as *Proteus* and other members of the family *Enterobacteriaceae*.[2] The medium employs **Trypticase** Soy Agar supplemented with additional agar, yeast extract, vitamin K_1, hemin, cystine, 5% sheep blood and phenylethyl alcohol.

CDC Anaerobe Laked Blood Agar with Kanamycin and Vancomycin was formulated as an enriched, selective medium for the isolation and cultivation of *Prevotella melaninogenica, Fusobacterium necrophorum, Fusobacterium nucleatum* and other fastidious, obligately anaerobic, gram-negative bacilli, from clinical materials containing mixed populations.[1,3] The medium employs **Trypticase** Soy Agar supplemented with additional agar, yeast extract, vitamin K_1, hemin, cystine, 5% sheep blood, kanamycin and vancomycin. The combination of kanamycin and vancomycin for use in selective isolation of gram-negative anaerobes was first described by Finegold et. al.[7] Vancomycin, however, may inhibit *Porphyromonas asaccharolytica*.[8] This medium is similar to CDC Anaerobe 5% Sheep Blood Agar and Kanamycin and Vancomycin except that the blood has been laked, by subjecting it to three freeze-thaw cycles, for improved pigmentation of the *P. melaninogenica-P. asaccharolytica group*.[9]

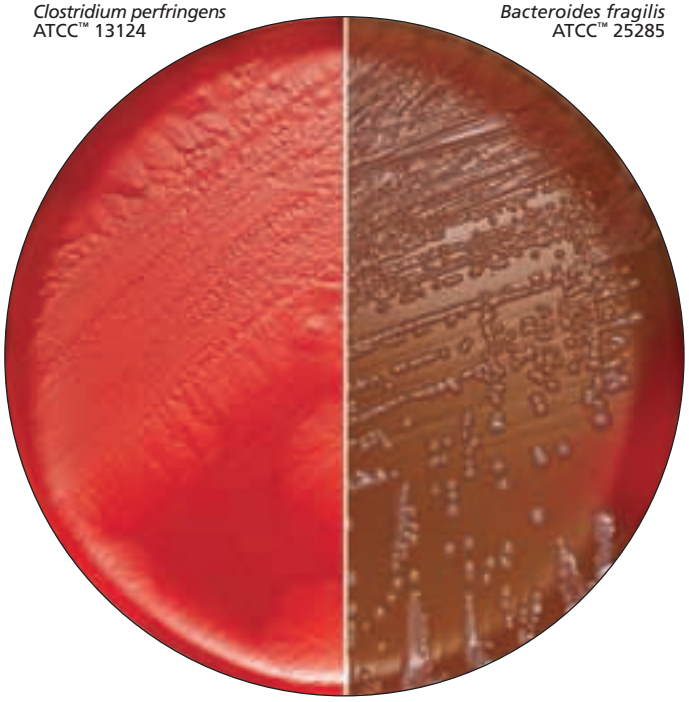
Principles of the Procedure

CDC Anaerobe 5% Sheep Blood Agar is a highly nutritious medium due to its content of peptones, yeast extract, hemin, vitamin K_1 and sheep blood. The peptones provide nitrogenous growth factors, carbon, sulfur and trace ingredients. Yeast extract is an important source of B vitamins. Sodium chloride

CDC Anaerobe 5% Sheep Blood Agar

Clostridium perfringens ATCC™ 13124

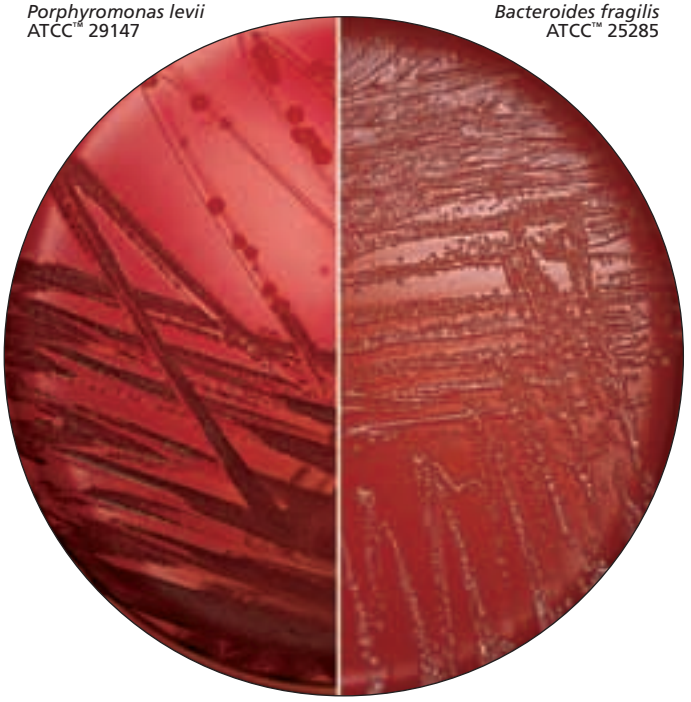
Bacteroides fragilis ATCC™ 25285



CDC Anaerobe Agar with Laked Sheep Blood and KV

Porphyromonas levii ATCC™ 29147

Bacteroides fragilis ATCC™ 25285



CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol (PEA)

Peptostreptococcus anaerobius ATCC™ 27337

maintains osmotic equilibrium. Sheep blood constituents, hemin, cystine and vitamin K_1 provide growth factors required by certain obligate anaerobes.[3-6,10,11]

The addition of the antimicrobial agents, kanamycin and vancomycin, renders the medium selective for gram-negative microorganisms. The kanamycin inhibits protein synthesis in susceptible organisms, whereas the vancomycin inhibits gram-positive bacteria by interfering with cell wall synthesis.[12]

Selectivity is achieved through the addition of phenylethyl alcohol which reduces the growth of facultatively anaerobic, gram-negative bacteria without inhibiting growth of obligately anaerobic bacteria.[13]

The addition of laked sheep blood to CDC Anaerobe Agar improves pigmentation of the *Prevotella*, *Porphyromonas*, and pigmented *Bacteroides* species.[12]

Divided Petri dishes containing CDC Anaerobe 5% Sheep Blood Agar in each half enable two specimens to be streaked on one plate. Combinations of selective media are also provided in divided dishes.

Procedure

Streak the specimen as soon as possible after it is received in the laboratory. Minimize exposure to air. With liquid specimens, media should be inoculated with one drop of the specimen. Tissue specimens should be minced and then ground in sterile broth, such as **BBL** Enriched Thioglycollate Medium, before inoculation. Inoculation is then performed as for liquid specimens. Swab specimens may be rolled onto the first quadrant of plated media and then used to inoculate liquid media. Alternatively, the swab may be "scrubbed" in a small volume

of reduced broth and the broth used to inoculate media as performed with liquid specimens.

These media should be reduced immediately prior to inoculation by placing under anaerobic conditions for 18-24 hours.[3] An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak**™ EZ anaerobic systems or an alternative anaerobic system.[14]

Plated media should be inoculated using the streak plate method in order to obtain pure cultures from specimens containing mixed flora.

An enrichment broth, such as **BBL** Enriched Thioglycollate Medium, should be inoculated at the same time as the primary isolation plates.

Incubate immediately under anaerobic conditions or place in a holding jar flushed with oxygen free gas(es) until sufficient plates are accumulated (but no longer than 3 hours).[14] Incubation should be at 35 ± 2°C for at least 48 hours and up to 7 days.

Expected Results

Examine colonies using a dissecting microscope and a long-wave UV lamp (colonies of the pigmenting *Porphyromonas-Prevotella* species should fluoresce orange to brick-red under UV light). Fluorescence is visible before pigmentation.

In order to determine the relationship to oxygen of each colony type present on anaerobic solid media, inoculate the following media:[15]

1. One anaerobe blood agar plate to be incubated anaerobically.
2. One aerobic blood agar (or chocolate agar) plate to be incubated in an aerobic atmosphere enriched with carbon dioxide. The chocolate agar is particularly needed to distinguish nutritionally-fastidious *Haemophilus* species and other bacteria which will grow on anaerobe blood agar incubated anaerobically and on chocolate agar under increased carbon dioxide tension but which fail to grow on blood agar in the presence of carbon dioxide or in the air.
3. One aerobic blood agar plate to be incubated aerobically without added carbon dioxide.
4. Tubes of Enriched Thioglycollate Medium and/or Cooked Meat Medium and a tube of Peptone Yeast Extract Glucose Broth.

Incubate all cultures at 35 ± 2°C for a minimum of 24 hours and up to 7 days.

Record the relationship to oxygen as either obligate anaerobe or nonanaerobe (aerotolerant anaerobe, microaerophilic, or facultative anaerobe).[15]

Colonies of the type(s) which proved to be obligate anaerobes can be further studied using the corresponding broth cultures.

Organisms failing to grow on the aerobic subculture plates may be presumed to be obligately anaerobic in terms of their oxygen requirements.

References

1. Dowell. 1975. *In* Balows (ed.), Clinical microbiology. How to start and when to stop Charles C. Thomas, Springfield, Ill.
2. Dowell, Lombard, Thompson and Armfield. 1977. Media for isolation, characterization, and identification of obligately anaerobic bacteria. CDC laboratory manual. Center for Disease Control, Atlanta, Ga.
3. Dowell and Hawkins. 1987. Laboratory methods in anaerobic bacteriology. CDC laboratory manual. HHS Publication (CDC) 87-8272. Center for Disease Control, Atlanta, Ga.
4. Forbes and Granato. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. Rodloff, Applebaum and Zabransky. 1991. Cumitech 5A, Practical anaerobic bacteriology. Coord. ed., Rodloff. American Society for Microbiology, Washington, D.C.
6. Star, Killgore and Dowell. 1971 Appl. Microbiol. *22*:655.
7. Finegold, Miller, and Posnick. 1965. Ernahrungsforschung. *10*:517.
8. van Winkelhoff and de Graaf. 1983. J. Clin. Microbiol. *18*:1282.
9. Finegold and Citron. 1980. *In* Lennette, Balows, Hausler and Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
10. Gibbons and MacDonald. 1960. J. Bacteriol. *80*:164.
11. Wilkins, Chalgren, Jimenez-Ulate, Drake and Johnson. 1976. J. Clin. Microbiol. *3*:359.
12. Estevez. 1984. Lab. Med. *15*:258.
13. Dowell, Hill and Altemeir. 1964. J. Bacteriol. *88*:1811.
14. Martin. 1971. Appl. Microbiol. *22*:1168.
15. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler, and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ CDC Anaerobe 5% Sheep Blood Agar

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	221733	Prepared Plates – Pkg. of 20*
	221734	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	256506	Prepared Plates – Pkg. of 20*

Japan

Cat. No.	251733	Prepared Plates – Pkg. of 20*
	251584	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*

BBL™ CDC Anaerobe 5% Sheep Blood Agar with Kanamycin and Vancomycin (KV)

CMPH2 MCM9

Cat. No.	221735	Prepared Plates – Pkg. of 20*
	221736	Prepared Plates – Ctn. of 100*

BBL™ CDC Anaerobe 5% Sheep Blood Agar with Phenylethyl Alcohol (PEA)

BS12 CMPH2 MCM9

Cat. No.	221739	Prepared Plates – Pkg. of 20*

BBL™ CDC Anaerobe Agar with Laked Sheep Blood and KV

CMPH2 MCM9

Cat. No.	221846	Prepared Plates – Pkg. of 20*

BBL™ CDC Anaerobe 5% Sheep Blood Agar// CDC Anaerobe 5% Sheep Blood Agar with PEA

Cat. No.	297646	Prepared Bi-Plate Dishes – Pkg. of 20*

BBL™ CDC Anaerobe 5% Sheep Blood Agar with KV// CDC Anaerobe 5% Sheep Blood Agar with PEA

Cat. No.	297004	Prepared Bi-Plate Dishes – Pkg. of 20*

BBL™ CDC Anaerobe 5% Sheep Blood Agar with PEA// Anaerobe Laked Sheep Blood Agar with KV

Cat. No.	299611	Prepared **I Plate**™ Dishes – Pkg. of 20*

**Store at 2-8°C.*

CIN Agar
Yersinia Selective Agar Base
Yersinia Antimicrobic Supplement CN

Intended Use

CIN (cefsulodin-Irgasan™*-novobiocin) Agar supplemented with cefsulodin and novobiocin is a differential and selective medium used in qualitative procedures for the isolation of *Yersinia enterocolitica* from a variety of clinical and nonclinical specimens.

**Irgasan is a trademark of Ciba-Geigy.*

Summary and Explanation

CIN Agar, also known as Yersinia Selective Agar, was first described by Schiemann as an alternative to MacConkey Agar and other commonly used media for isolation of *Y. enterocolitica*, a causative agent of gastroenteritis.[1] CIN Agar has been found to be far superior to MacConkey, SS, CAL or Y agars for the recovery of *Y. enterocolitica*.[2]

Principles of the Procedure

Fermentation of mannitol in the presence of neutral red results in a characteristic "bull's-eye" colony, colorless with red center. Selective inhibition of gram-negative and gram-positive organisms is obtained by means of crystal violet, sodium desoxycholate and Irgasan (triclosan). Supplementation with Yersinia Antimicrobic Supplement CN (cefsulodin and novobiocin) improves inhibition of normal enteric organisms. Organisms that do not metabolize mannitol to acid end products will form colorless, translucent colonies.

Formulae

Difco™ Yersinia Selective Agar Base

Approximate Formula* Per Liter

Peptone	17.0	g
Proteose Peptone	3.0	g
Yeast Extract	2.0	g
Mannitol	20.0	g
Sodium Pyruvate	2.0	g
Sodium Chloride	1.0	g
Magnesium Sulfate Heptahydrate	10.0	mg
Sodium Desoxycholate	0.5	g
Sodium Cholate	0.5	g
Irgasan™	4.0	mg
Agar	13.5	g
Crystal Violet	1.0	mg
Neutral Red	30.0	mg

Difco™ Yersinia Antimicrobic Supplement CN

Formula Per 10 mL Vial

Cefsulodin	4.0	mg
Novobiocin	2.5	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

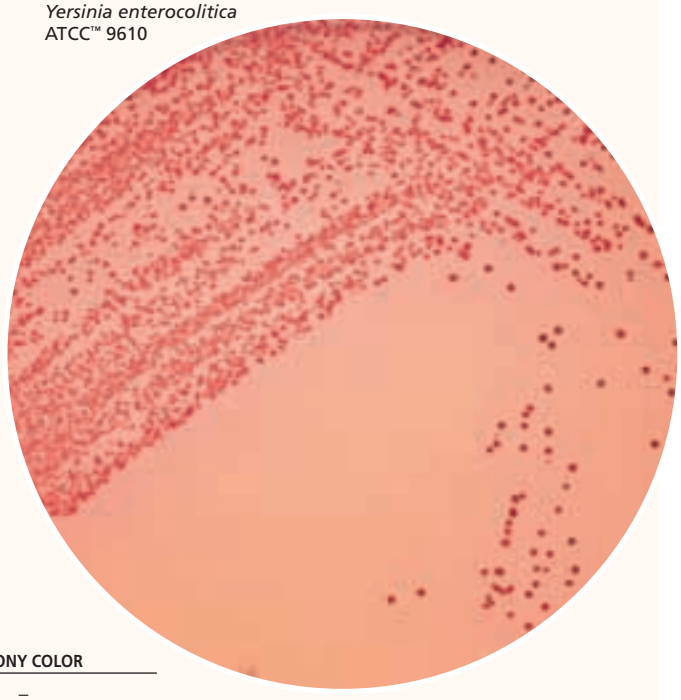
Difco™ Yersinia Selective Agar Base

Dehydrated Appearance:	Light beige to light pinkish beige, free-flowing, homogeneous.
Solution:	5.95% solution, soluble in purified water upon boiling. Solution is reddish-purple, very slightly to slightly opalescent.
Prepared Appearance:	Reddish-orange, very slightly to slightly opalescent.
Reaction of 5.95% Solution at 25°C:	pH 7.4 ± 0.2

Difco™ Yersinia Antimicrobic Supplement CN

Dehydrated Appearance:	Lyophilized, white, homogeneous cake.
Solution:	Soluble on rehydration with 10 mL purified water. Solution is colorless, clear.

Yersinia enterocolitica
ATCC™ 9610



Cultural Response

Difco™ Yersinia Selective Agar Base

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^3	Inhibition	–
Escherichia coli	25922	10^3	Inhibition	–
Proteus mirabilis	12453	10^3	Inhibition	–
Pseudomonas aeruginosa	27853	10^3	Inhibition	–
Yersinia enterocolitica	9610	10^2	Good	Colorless with dark pink centers, may have bile precipitate

Directions for Preparation from Dehydrated Product

Yersinia Antimicrobic Supplement CN

1. To rehydrate the supplement, aseptically add 10 mL of purified water to the vial.
2. Invert the vial several times to dissolve the powder.

Yersinia Selective Agar (CIN Agar)

1. Suspend 59.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. For the preparation of CIN Agar, cool to 45-50°C and aseptically add 10 mL of rehydrated Yersinia Antimicrobic Supplement CN. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 25°C for 24-48 hours.

If a cold enrichment procedure is desired, inoculate the specimen into phosphate buffered saline and hold at 4°C for up to 21 days.[3,4] Periodically subculture onto plates of CIN Agar, streaking for isolation. Incubate plates as stated above.

Expected Results

Typical *Y. enterocolitica* colonies will have deep-red centers surrounded by a transparent border giving the appearance of a "bull's eye."

Growth of non-*Yersinia* organisms is markedly to completely inhibited.

Limitation of the Procedure

Although certain strains of *Yersinia* can be recovered by direct plating, others may require cold enrichment (4°C) in phosphate-buffered saline.[3] However, cold enrichment may not be practical because of the long incubation time and because it selects for nonpathogenic *Yersinia* species.[4]

References

1. Schiemann. 1979. Can. J. Microbiol. *25*:1298.
2. Head, Whitty and Ratnam. 1982. J. Clin. Microbiol. *16*:615.
3. Weissfeld and Sonnenwirth. 1982. J. Clin. Microbiol. *15*:508.
4. Wanger. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller, (ed.), Manual of clinical microbiology. 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Yersinia Selective Agar Base

BAM COMPF ISO SMD

Cat. No. 218172 Dehydrated – 500 g

Difco™ Yersinia Antimicrobic Supplement CN

BAM COMPF ISO SMD

Cat. No. 231961 Vial – 6 × 10 mL*

BBL™ CIN Agar (Yersinia Selective Agar)

BAM BS12 CMPH2 COMPF ISO MCM9 SMD

United States and Canada

Cat. No. 221848 Prepared Plates – Pkg. of 10*
299579 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254056 Prepared Plates – Pkg. of 20*
254088 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 251139 Prepared Plates – Pkg. of 20*

Mexico

Cat. No. 230550 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

CLED Agar

Intended Use

CLED Agar is used for the isolation, enumeration and presumptive identification of microorganisms from urine.

Summary and Explanation

In 1960, Sandys reported on the development of a new method of preventing the swarming of *Proteus* on solid media by restricting the electrolytes in the culture medium.[1] Previous chemical methods used to inhibit swarming by *Proteus* included the addition of chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid and sulfonamides to the culture medium.[1]

This electrolyte-deficient medium of Sandys was modified by Mackey and Sandys[2] for use in urine culture by substituting lactose and sucrose for the mannitol and increasing the concentrations of the bromthymol blue indicator and of the agar. These two investigators further modified the medium by the incorporation of cystine in order to enhance the growth of cystine-dependent "dwarf colony" coliforms and by deletion of sucrose.[3] They designated the new medium as Cystine-Lactose-Electrolyte-Deficient (CLED) medium and reported it to be ideal for dip-inoculum techniques and for urinary bacteriology in general.

CLED Agar is recommended for use in plates or in urine dipsticks for detecting significant bacteriuria by quantitative culture of urine. For reliable results, inoculation of the medium must occur as soon after collection as possible. Confluent or semiconfluent growth of bacteria will occur on the surface of the dipstick medium when bacterial counts are greater than 10^5 per mL of urine, as confirmed by plates inoculated by the calibrated-loop or duplicate-dilution pour-plate methods.[4] Once the medium has been inoculated by immersion of the dipstick or by pouring the urine over the surface of the medium if only a small volume is available, the dipstick may be held 48 hours or longer, refrigerated or at room

User Quality Control

Identity Specifications

BBL™ CLED Agar

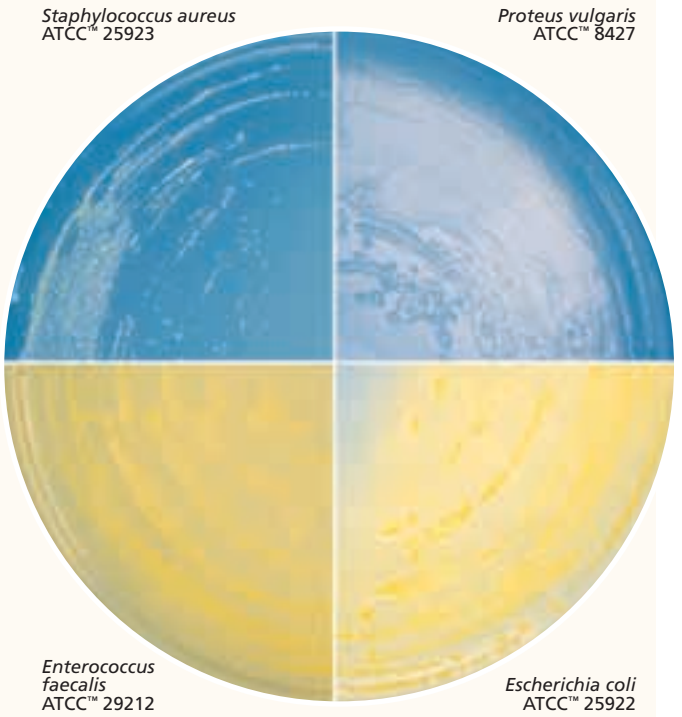
Dehydrated Appearance:	Fine, homogenous, free of extraneous material.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is medium, yellow green to blue green, clear to slightly hazy, with up to a large amount of minute suspended insolubles.
Prepared Appearance:	Medium, yellow green to blue green, clear to slightly hazy.
Reaction of 3.6% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ CLED Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Enterococcus faecalis	29212	10^3-10^4	Good	Yellow
Escherichia coli	25922	10^3-10^4	Good	Yellow
Klebsiella pneumoniae	33495	10^3-10^4	Good	With or without green to yellow reaction
Pseudomonas aeruginosa	10145	10^3-10^4	Good	With or without blue reaction
Staphylococcus aureus	25923	10^3-10^4	Good	Yellow
Proteus vulgaris	8427	10^3-10^4	Good	Blue



temperature until received in the laboratory. On receipt, the dipstick should be incubated at 35 ± 2°C for 18-24 hours, to allow colonies to develop on the medium.

Principles of the Procedure

The nutrients in CLED Agar are supplied by peptones, pancreatic digests of gelatin and casein, and beef extract. Lactose is included to provide an energy source for organisms capable of utilizing it by a fermentative mechanism. The cystine permits the growth of "dwarf colony" coliforms. Bromthymol blue is used as a pH indicator to differentiate lactose fermenters from lactose nonfermenters. Organisms that ferment lactose will lower the pH and change the color of the medium from green to yellow. Electrolyte sources are reduced in order to restrict the swarming of *Proteus* species.

Bacteriuria is determined by inoculating the surface of an agar medium using 0.1 mL of a 10^{-2} dilution of the urine sample or using a calibrated loop (0.001 mL) of the undiluted sample.[5] Current guidelines are that for a single isolate a density of $>10^5$ CFU/mL indicates infection, $<10^4$ CFU/mL indicates urethral or vaginal contamination, and between 10^4 and 10^5 CFU/mL needs to be evaluated based on clinical information.[6]

Formula

BBL™ CLED Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	4.0	g
Pancreatic Digest of Casein	4.0	g
Beef Extract	3.0	g
Lactose	10.0	g
L-Cystine	128.0	mg
Bromthymol Blue	0.02	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the medium as soon as possible after the specimen is received in the laboratory. It is recommended that quantitative methods be used for culturing urine specimens.[5] Incubate at 35 ± 2°C for 24-48 hours.

Expected Results

Count the number of colonies on the plate or dipstick. Multiply by an appropriate number to convert the count to CFU per mL of sample.

Contaminant bacteria usually appear in low numbers which vary in colonial morphology. Urinary pathogens will usually yield high counts having uniform colonial morphology and should be subcultured directly to routine media for identification and susceptibility testing.[5,7]

Typical colonial morphology on CLED Agar is as follows:

Escherichia coli	Yellow colonies, opaque, center slightly deeper yellow
Klebsiella	Yellow to whitish-blue colonies, extremely mucoid
Proteus	Translucent blue colonies
Pseudomonas aeruginosa	Green colonies with typical matted surface and rough periphery
Enterococci	Small yellow colonies, about 0.5 mm in diameter
Staphylococcus aureus	Deep yellow colonies, uniform in color
Staphylococci coagulase-negative	Pale yellow colonies, more opaque than *E. faecalis*

Limitations of the Procedure

Factors that may cause urine counts from infected patients to be low include: rapid rate of urine flow, prior initiation of antimicrobial therapy, a urine pH of less than 5 and a specific gravity of less than 1.003.[7]

References

1. Sandys. 1960. J. Med. Lab. Technol. *17*:224.
2. Mackey and Sandys. 1965. Br. Med. J. *2*:1286.
3. Mackey and Sandys. 1966. Br. Med. J. *1*:1173.
4. Benner. 1970. Appl. Microbiol. *19*:409.
5. Barry, Smith and Turck. 1975. Cumitech 2, Laboratory diagnosis of urinary tract infections. Coord. ed., Gavan. American Society for Microbiology, Washington, D.C.
6. Clarridge, Pezzlo and Vosti. 1987. Cumitech 2A, Laboratory diagnosis of urinary tract infections. Coordinating ed., Weissfeld. American Society for Microbiology, Washington, D.C.
7. Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.

Availability

BBL™ CLED Agar

Cat. No. 212218 Dehydrated – 500 g

United States and Canada

Cat. No. 221850 Prepared Plates – Pkg. of 10*

Europe

Cat. No. 254003 Prepared Plates – Pkg. of 20*
254070 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 251953 Prepared Plates – Pkg. of 20*
251530 Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*

CTA Agar

Intended Use

CTA Agar is primarily used for carbohydrate fermentation tests with corynebacteria and especially for differentiation of *C. diphtheriae* from related species.

Summary and Explanation

CTA Medium™, a semi-solid formulation, was developed by Vera and is widely used for fermentation and motility determinations by a wide variety of microorganisms.[1] CTA Agar is the solid form of **CTA Medium** and, when employed as a plated medium and used in conjunction with **BBL**™ **Taxo**™ carbohydrate discs, is useful in the speciation of *Corynebacterium* isolates of medical importance.[2] Supplemented with carbohydrates and prepared as slants, it is used for the differentiation of *Neisseria* species.[3]

Principles of the Procedure

CTA Agar utilizes peptone as a carbohydrate-free source of nutrients. Inorganic salts are included in order to supply essential ions. Phenol red is an indicator of pH changes in the medium surrounding the **Taxo** carbohydrates discs, which are applied to the surface of inoculated plates.

Formula

BBL™ CTA Agar

Approximate Formula* Per Liter

L-Cystine	0.5	g
Pancreatic Digest of Casein	20.0	g
Agar	14.0	g
Sodium Chloride	5.0	g
Sodium Sulfite	0.5	g
Phenol Red	17.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 118°C for 15 minutes.
4. If desired, add 2 drops of sterile rabbit serum per tube prior to solidification in order to enhance the recovery of *C. diphtheriae*.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate a pure culture of the organism onto the surface of the plated medium using a swab technique to inoculate the entire surface. **Taxo** carbohydrate discs are then applied to the agar surface using no more than four discs per plate.

User Quality Control

Identity Specifications

BBL™ CTA Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is medium, orange-red to red-rose, clear to slightly hazy.
Prepared Appearance:	Orange-red to red-rose, slightly hazy.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ CTA Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY
Corynebacterium diphtheriae	11913	Growth
Corynebacterium pseudodiphtheriticum	10700	Growth

Incubate plates for 18-48 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Typical diphtheria bacilli ferment dextrose and maltose, but not sucrose.

Typical carbohydrate reactions for selected corynebacteria on CTA Agar plates containing **Taxo** carbohydrate discs are as follows:

CORYNEBACTERIUM SPECIES	DEXTROSE	MALTOSE	SUCROSE
C. diphtheriae	+	+	–
C. pseudodiphtheriticum	–	–	–
C. xerosis	+	+	+
C. jeikeium	+	v	–

+ = acid (yellow zone reaction)
– = no acid produced
v = variable reaction

Current schemes recommended for the identification of medically significant corynebacteria include carbohydrate utilization as part of the testing regimen. Appropriate references should be consulted for a discussion of the other tests, which enable a definitive identification of the above-named organisms as well as other clinically important species of corynebacteria.[4,5]

References

1. Vera. 1948. J. Bacteriol. *55*:531.
2. Alberti, Ortali and Turia. 1965. Ann. 1st. Superiore di Sanita. *1*:349.
3. Morello, Janda and Doern. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.

Availability

BBL™ CTA Agar

Cat. No. 211094 Dehydrated – 500 g

CTA Medium™ • Cystine Tryptic Agar
CTA Medium™ with Carbohydrates

Intended Use

Cystine Tryptic Agar and **CTA Medium** (Cystine **Trypticase**™ Agar Medium) are for the maintenance of microorganisms, as well as for the detection of bacterial motility and, with added carbohydrate, for fermentation reactions of fastidious microorganisms; i.e., *Neisseria*, pneumococci, streptococci and nonsporeforming anaerobes.

Summary and Explanation

This formulation was developed by Vera as a simple semi-solid medium for the identification and maintenance of the gonococcus and other bacteria.[1]

Without carbohydrates, it can be used for maintenance of cultures, including fastidious organisms, for extended periods when stored at appropriate temperatures.

With the appropriate carbohydrate, it is recommended for the differentiation of fastidious organisms by means of fermentation reactions. In the semisolid agar, acid reactions are easily detected because the acid formed is not immediately diffused throughout the entire culture as in a broth. When no fermentable carbohydrate is present, most cultures show an alkaline shift.

Motility can be readily detected in the semisolid medium.[2] Stab cultures show growth out from the line of inoculation. Nonmotile organisms grow in the inoculated area, while the surrounding area remains clear.

BBL™ Taxo™ carbohydrate discs can be selected and added, as needed, to tubes of plain **CTA Medium** when fermentation reactions are to be determined.

For clostridia, bacilli, common micrococci, enteric bacilli and other organisms not generally considered to be nutritionally fastidious, the use of **Trypticase** Agar Base is recommended instead of this formulation.

Principles of the Procedure

The medium contains cystine and peptone to supply the nutrients necessary to support the growth of fastidious microorganisms.

Carbohydrate fermentation is detected by a visible color change of the medium due to the incorporation of the pH indicator dye, phenol red. When the carbohydrate present is metabolized by the organism, organic acids are produced and the medium becomes acidified. However, the peptone present in the medium is also degraded by the bacteria present and yields substances that are alkaline in pH.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Cystine Tryptic Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	2.85% solution, soluble in purified water upon boiling. Solution is red, very slightly opalescent.
Prepared Appearance:	Red, very slightly opalescent.
Reaction of 2.85% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Cystine Tryptic Agar

Prepare the medium per label directions without and with 0.5% dextrose. Inoculate tubes with fresh broth cultures (Neisseria from chocolate agar) by straight stab and incubate with caps tightened at 35 ± 2°C for 18-48 hours (up to 72 hours if necessary).

ORGANISM	ATCC™	MOTILITY	ACID PRODUCTION WITH DEXTROSE
Corynebacterium diphtheriae biotype *mitis*	8024	–	+
Escherichia coli	25922	+	+
Neisseria gonorrhoeae	43070	–	+

Identity Specifications

BBL™ CTA Medium™

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.85% solution, soluble in purified water upon boiling. Solution is light to medium, red-orange to orange-red to red-rose, clear to slightly hazy.
Prepared Appearance:	Red-orange to red-rose, slightly hazy.
Reaction of 2.85% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ CTA Medium™

Prepare the medium per label directions (without added carbohydrate). Inoculate tubes with fresh broth cultures (*Neisseria* from chocolate agar) by straight stab and incubate at 35 ± 2°C under appropriate atmospheric conditions (*Neisseria* with tightened caps) for 72 hours.

ORGANISM	ATCC™	RECOVERY	MOTILITY
Corynebacterium pseudodiphtheriticum	10700	Good	–
Enterococcus faecalis	29212	Good	–
Listeria monocytogenes	19115	Good	+
Neisseria gonorrhoeae	19424	Good	–
Neisseria meningitidis	13090	Good	–
Staphylococcus aureus	6538P	Good	–

The phenol red indicator changes from reddish-orange to yellow when the amount of acid produced by carbohydrate fermentation is greater than the alkaline end products of peptone degradation. The color change with phenol red occurs around pH 6.8, near the original pH of the medium.

Formulae

Difco™ Cystine Tryptic Agar

Approximate Formula* Per Liter

Tryptose	20.0 g
L-Cystine	0.5 g
Sodium Chloride	5.0 g
Sodium Sulfite	0.5 g
Agar	2.5 g
Phenol Red	17.0 mg

BBL™ CTA Medium™

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0 g
L-Cystine	0.5 g
Sodium Chloride	5.0 g
Sodium Sulfite	0.5 g
Agar	2.5 g
Phenol Red	17.0 mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Cystine Tryptic Agar

1. Suspend 28.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at not over 118°C for 15 minutes.
4. To prepare fermentation medium, add 5-10 g of carbohydrate before autoclaving or dissolve medium in 900 mL water, autoclave, and aseptically add 100 mL sterile 5-10% carbohydrate solution.
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ CTA Medium™

1. Suspend 28.5 g of the powder in 1 L of purified water. Add carbohydrate (0.5 to 1.0%) if desired, and adjust the pH if necessary. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute or until solution is complete.
3. Tube and autoclave at not over 118°C for 15 minutes. Cool in the upright position.
4. Store at room temperature. Do not refrigerate unless in tightly closed, screw-capped tubes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Loosen caps, boil, tighten caps and cool before use.
2. Remove fresh colony growth from the surface of a suitable culture medium; e.g., Chocolate Agar, not from a selective, primary isolation plate.[3]

3. For fermentation tests with members of the genus *Neisseria*, only the surface of the tubed medium is inoculated. For facultative organisms, such as streptococci and strictly anaerobic organisms, inoculate by stabbing the center of the medium with an inoculating needle to about 1/2 the depth of the medium.
4. Repeat for each tube to be inoculated.
5. Incubate at 35 ± 2°C with loosened caps aerobically or anaerobically depending upon the organisms being tested; *Neisseria* should be incubated with tight caps[4] especially if tubes must be incubated in a CO_2 incubator,[5,6] or with loose caps in a non-CO_2 incubator.[7,8] Examine periodically up to 24 hours for growth (turbidity), evidence of motility, and acid production in carbohydrate-containing medium (yellow color in upper layer of medium). A few strains may require incubation for up to 48-72 hours.[9]
6. Many fastidious organisms, including *Neisseria, Pasteurella*, streptococci, *Brucella*, corynebacteria and vibrios, may be readily cultivated in this medium, no added carbon dioxide, serum or other enrichments being required.
7. For more rapid growth and also for more rapid fermentation reactions, anaerobic cultures preferably should be incubated in the presence of carbon dioxide as well as hydrogen or nitrogen. Some strict anaerobes fail to grow or grow poorly in the absence of carbon dioxide.

Expected Results

A yellow color either in the upper one-third or throughout the medium indicates acid production; i.e., fermentation of the carbohydrate. A red (alkaline) to orange (neutral) color indicates that the carbohydrate has not been degraded and that only the peptone has been utilized. Inoculated medium (without carbohydrate) also exhibits a red to orange color.

Motile organisms show growth out from the line of stab-inoculation. Nonmotile organisms only grow along the stab line with the surrounding agar remaining clear.

Limitations of the Procedure

1. CTA requires a heavy inoculum.[10]
2. Prolonged incubation may lead to changes in pH indicator or abnormal lactose/sucrose reactions with *Neisseria* pathogens.[11,12]
3. *Neisseria* species usually produce acid only in the area of stabs (upper third). If there is a strong acid (yellow color) throughout the medium, a contaminating organism may be present. If in doubt about a tube containing a *Neisseria* species, a Gram stain and oxidase test should be performed on the growth.[10]

References

1. Vera. 1948. J. Bacteriol. *55*:531.
2. Vera and Petran. 1954. Bull. Nat. Assoc. Clin. Labs. *5*:90.
3. Morello, Janda and Doern. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
4. Kellogg. 1974. *In* Lennette, Spaulding and Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
5. Yu and Washington. 1985. *In* Washington (ed.), Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
6. Morse and Knapp. 1987. *In* Wentworth (ed.), Diagnostic procedures for bacterial infections, 7th ed. American Public Health Association, Washington, D.C.
7. Center for Disease Control. 1978. Laboratory methods in clinical bacteriology. CDC, Atlanta, Ga.
8. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
9. Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.
10. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
11. Faur, Weisburd and Wilson. 1975. J. Clin. Microbiol. *1*:294.
12. Applebaum and Lawrence. 1979. J. Clin. Microbiol. *9*:598.

Availability

Difco™ Cystine Tryptic Agar

AOAC

Cat. No.	252310	Dehydrated – 500 g

BBL™ CTA Medium™

AOAC

Cat. No.	211096	Dehydrated – 500 g
	221631	Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
	221632	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*

BBL™ CTA Medium™ with Carbohydrates

Cat. No.	297731	Prepared Tubes with Arabinose – Pkg. of 10*
	297732	Prepared Tubes with Cellobiose – Pkg. of 10*
	221633	Prepared Tubes with Dextrose – Pkg. of 10*
	221634	Prepared Tubes with Dextrose – Ctn. of 100*
	296001	Prepared Tubes with Fructose – Pkg. of 10*
	221635	Prepared Tubes with Lactose – Pkg. of 10*
	221637	Prepared Tubes with Maltose – Pkg. of 10*
	221639	Prepared Tubes with Mannitol – Pkg. of 10*
	297101	Prepared Tubes with Raffinose – Pkg. of 10*
	297102	Prepared Tubes with Rhamnose – Pkg. of 10*
	221641	Prepared Tubes with Salicin – Pkg. of 10*
	221643	Prepared Tubes with Sorbitol – Pkg. of 10*
	296002	Prepared Tubes with Starch – Pkg. of 10*
	221645	Prepared Tubes with Sucrose – Pkg. of 10*
	297033	Prepared Tubes with Trehalose – Pkg. of 10*
	221647	Prepared Tubes with Xylose – Pkg. of 10*

**Store at 2-8°C.*

Campy-Cefex Agar

Intended Use

Campy-Cefex Agar* is a selective medium used for the primary isolation and cultivation of *Campylobacter* species, especially *C. jejuni* and *C. coli*, from poultry.

** U.S. Patent No. 5,891,709*

Summary and Explanation

In 1992, Stern et al. published on the development of Campy-Cefex Agar, a selective-differential medium for the isolation of *Campylobacter* species from chicken carcasses. Campy-Cefex Agar demonstrated easier differentiation of *C. jejuni* from other flora when compared to Campylobacter Cefoperazone Desoxycholate Agar and better selectivity than Campylobacter Brucella Agar (Campy BAP).[1]

In September 2005, Campy-Cefex Agar was adopted by the National Advisory Committee on Microbiological Criteria for Foods for the isolation of *Campylobacter* species from chicken carcasses.[2]

Principles of the Procedure

This medium consists of Brucella Agar, a general purpose medium that supports the growth of *Campylobacter* species. Laked horse blood provides additional nutrients. Antimicrobial agents are incorporated to suppress the growth of normal fecal flora that could mask the presence of *C. jejuni*. Cefoperazone is a cephalosporin antibiotic that suppresses the growth of gram-negative enteric bacilli and some gram-positive species. Cycloheximide is used to suppress the growth of fungi.

Sample Collection and Handling

For agrifood samples consult appropriate standard methods for details on sample preparation and processing according to sample type.[3,4]

Procedure

Inoculate the sample as soon as possible after it is received in the laboratory, by means of a swab, directly onto the agar surface and streak the plate for isolation. Incubate inoculated plates, protected from light, at 42°C in a reduced oxygen, increased carbon dioxide atmosphere. This atmosphere can be achieved by using the **BD GasPak**™ EZ Campy Container System with sachets or the **BD GasPak** EZ Campy Pouch System with sachets. Examine plates after 36-48 hours incubation.[1]

Expected Results

Colonies of *Campylobacter* appear translucent. Direct examination using phase-contrast microscopy (x1000) can be used to confirm typical morphology and motility – curved or spiral-shaped bacterial rods that may demonstrate a rapid corkscrew-like movement. Suspect colonies that demonstrate the described colonial and microscopic morphology, and are catalase and oxidase positive, can be presumptively identified as *Campylobacter* species.[1,5]

Limitations of the Procedure

1. Since *C. jejuni* is thermophilic, it is important to incubate the plates at 42°C; otherwise growth will be delayed. Also, the higher temperature improves selectivity by inhibiting the normal flora.
2. For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[2-4]

References

1. Stern, Wojton and Kwiatek. 1992. J. Food Protect. *55*:514.
2. NACMCF Executive Secretariat. 2007. Analytical utility of *Campylobacter* methodologies. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C. J. Food Protect. *70*:241.
3. Ransom and Rose. 1998. Isolation, identification, and enumeration of *Campylobacter jejuni/coli* from meat and poultry products. *In* Microbiology laboratory guidebook, 3rd ed., Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C.
4. Hunt, Abeyta and Tran. 2001. Chapter 7 *Campylobacter*. *In* Bacteriological analytical manual, online. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Washington, D.C.
5. Stern and Pretanik. 2006. Counts of *Campylobacter* spp. on U.S. broiler carcasses. J. Food Protect. *69*:1034.

Availability

BBL™ Campy-Cefex Agar

Cat. No.	215221	Prepared Plates – Pkg. of 20*
	292487	Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*

Campylobacter Agars

Campylobacter Agar Base • Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood (Blaser) Campy CSM Agar • Campy CVA Agar • Skirrows Medium Campylobacter Antimicrobic Supplement Skirrow Camplyobacter Antimicrobic Supplement Blaser

Intended Use

Campylobacter Agar Base, when supplemented with blood or other additives and antimicrobial agents, is used for the primary isolation and cultivation of *Campylobacter jejuni* subsp. *jejuni* from human fecal specimens. Several prepared selective media formulations are provided for the same purpose.

Summary and Explanation

In 1972, Dekeyser et al. reported that *C. jejuni* was isolated from the feces of patients with diarrhea and acute gastroenteritis using a filtration technique and a blood-containing selective medium with antimicrobics to suppress the normal enteric flora.[1] Subsequently, Skirrow and other investigators reported similar blood-based selective media that differed in the numbers and types of antimicrobics.[2-6] Bolton et al. reported that charcoal can effectively replace the blood in selective media for campylobacters.[7]

In 1978, Blaser et al. reported success in isolating *C. jejuni* with a medium containing four antimicrobics incorporated into Brucella Agar supplemented with 10% defibrinated sheep blood.[3,4] Subsequently, cephalothin was incorporated to increase its ability to inhibit the normal bacterial flora associated with fecal specimens.[5]

In 1983, Reller et al. introduced an improved selective medium containing cefoperazone, vancomycin and ampho-

tericin B (CVA) for isolation of *C. jejuni*.[6] They reported that this combination of antimicrobial agents provided better inhibition of normal fecal flora for easier detection of *C. jejuni* than the selective blood agar plate developed previously.

Karmali et al., in 1986, evaluated a blood-free, charcoal-based selective medium (designated CSM) in parallel with a Skirrow-type selective medium containing lysed horse blood. They reported that the quality of *Campylobacter* growth on CSM (luxuriant growth with smooth and effuse colonies) was similar to that seen on blood-based media and was significantly more selective than Skirrow medium.[8]

Principles of the Procedure

These media support the growth of *Campylobacter* species due to their content of peptones, yeast extract and other digests, extracts and components specific for the individual formulations provided. *Campylobacter* isolation relies, in addition, on a medium's selectivity, which depends on the antimicrobial agents in the medium, a microaerophilic environment and the incubation temperature of 42°C, which suppresses the growth of most normal bacteria.[9]

The antimicrobial agents required to make Skirrow's and Blaser's formulations are provided as Campylobacter Antimicrobic Supplement Skirrow and Campylobacter Antimicrobic Supplement Blaser, respectively.

Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood supports the growth of *Campylobacter* species due to its content of peptones, dextrose, yeast extract and blood. The peptones supply nitrogenous compounds, carbon, sulfur and trace ingredients. Yeast extract is a source of the B-complex vitamins. Dextrose is utilized as an energy source. Sheep blood supplies additional nutrients. The incorporation of the antimicrobial agents (amphotericin B, cephalothin, polymyxin B, trimethoprim and vancomycin) suppresses the growth of the normal microbial flora in fecal specimens, thereby facilitating isolation of *C. jejuni*.

Skirrows Medium contains, in addition to the usual nutritional components, laked horse blood, which supplies the X factor (heme) and other growth requirements. Vancomycin inhibits gram-positive bacteria, polymyxin B inhibits most gram-negative bacilli except *Proteus* and trimethoprim is inhibitory for *Proteus* spp.

Campy CSM Agar consists of Columbia Agar Base supplemented with activated charcoal, hematin, sodium pyruvate and three antimicrobial agents (cefoperazone, cycloheximide and vancomycin). The charcoal, hematin and sodium pyruvate improve the aerotolerance of *Campylobacter* species; it has been suggested that these supplements act as quenching agents of photochemically-produced toxic oxygen derivatives.[8] Cefoperazone is a cephalosporin antibiotic that suppresses the growth of gram-negative enteric bacilli and some gram-positive species. Vancomycin is a glycopeptide antibiotic that inhibits many species of gram-positive bacteria. Cycloheximide is an antifungal agent.

Campy CVA Agar consists of Brucella Agar, a general-purpose medium that supports the growth of *Campylobacter* species. Defibrinated sheep blood provides additional nutrients. Antimicrobial agents are incorporated to suppress the growth of normal fecal flora that could mask the presence of *C. jejuni*. Cefoperazone is a cephalosporin antibiotic that suppresses the growth of gram-negative enteric bacilli and some gram-positive species. Vancomycin is a glycopeptide antibiotic that inhibits many species of gram-positive bacteria. Amphotericin B is an antifungal agent.

Formulae

Difco™ Campylobacter Agar Base

Approximate Formula* Per Liter		
Proteose Peptone No. 3	15.0	g
Liver Digest	2.5	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Agar	12.0	g

Difco™ Campylobacter Antimicrobic Supplement Skirrow

Formula Per 5 mL Vial		
Vancomycin	5.0	mg
Polymyxin B	1250.0	units
Trimethoprim	2.5	mg

Difco™ Campylobacter Antimicrobic Supplement Blaser

Formula Per 5 mL Vial		
Vancomycin	5.0	mg
Polymyxin B	1250.0	units
Trimethoprim	2.5	mg
Cephalothin	7.5	mg
Amphotericin B	1.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 39.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool the medium to 45-50°C.
4. Aseptically add 5-7% sterile lysed horse blood or 10% sterile defibrinated sheep blood. Mix thoroughly.
5. **To prepare Skirrow's medium:** aseptically rehydrate one vial of Campylobacter Antimicrobic Supplement Skirrow with 5 mL of sterile purified water. Rotate in an end-over-end motion to dissolve the contents completely. Store the rehydrated vials at 2-8°C. Use within 24 hours after rehydration.
   To prepare Blaser's medium: aseptically rehydrate one vial of Campylobacter Antimicrobic Supplement Blaser with 5 mL of sterile purified water. Rotate in an end-over-end motion to dissolve the contents completely. Store the rehydrated vials at 2-8°C. Use within 24 hours after rehydration.

   Aseptically add 1% of the desired antimicrobic supplement (10 mL of supplement to 1 L or 5 mL of supplement to 500 mL of medium base). Mix thoroughly, avoiding the formation of air bubbles and dispense into sterile Petri dishes.
6. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Campylobacter Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in purified water upon boiling. Solution is medium to dark amber, clear to slightly opalescent.
Prepared Appearance:	Plain – Medium to dark amber, very slightly to slightly opalescent. With 10% sheep blood – Cherry red, opaque.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

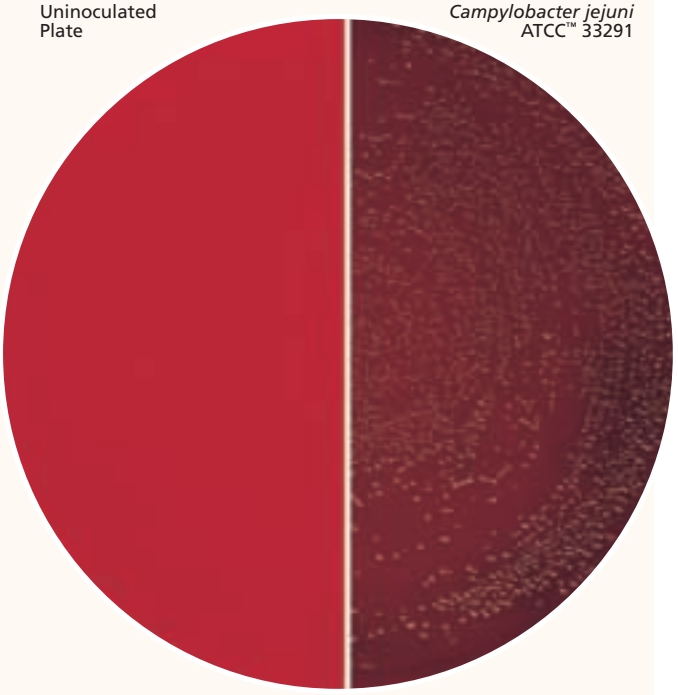
Cultural Response

Difco™ Campylobacter Agar Base

Prepare the medium per label directions; e.g., with 10% sterile defibrinated sheep blood and antimicrobic supplements (Skirrow or Blaser). Inoculate and incubate at 42°C under microaerophilic conditions for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY SKIRROW	RECOVERY BLASER
Campylobacter jejuni subsp. *jejuni*	29428	10^2-10^3	Good	Good
Campylobacter jejuni subsp. *jejuni*	33291	10^2-10^3	Good	Good
Candida albicans	10231	10^3	Good	Inhibition
Enterococcus faecalis	33186	10^3	Inhibition	Inhibition
Escherichia coli	25922	10^3	Inhibition	Inhibition

Campylobacter Agar Base



Campy CSM Agar

Campy CVA Agar

Procedure

Use standard procedures to obtain isolated colonies from specimens. If immediate inoculation of a *Campylobacter* agar cannot be performed, the use of a suitable holding medium (e.g., Campylobacter Thioglycollate Medium with 5 Antimicrobics) is recommended. Incubate inoculated plates at 42°C in an atmosphere conducive to the primary isolation and cultivation of microaerophilic organisms. This atmosphere can be achieved by using one **BBL™ CampyPak™ Plus** disposable gas generator envelope in a **GasPak™** 100 jar, three envelopes in a **GasPak** 150 jar or using the **Bio-Bag™** Type Cfj or **GasPak** EZ Campy systems. Alternatively, the atmosphere can be achieved using evacuation of **GasPak** vented jars and replacement with cylinder gases, or by using the Fortner principle.[10]

Examine plates at 24 and 48 hours.

NOTE: If plates are to be examined after 24 hours of incubation, treat plates as if they were anaerobic cultures; i.e., examine plates quickly and place them back into a reduced oxygen atmosphere immediately after examination.

Expected Results

Campylobacter jejuni produces two types of colonies. One is small, raised, grayish-brown, smooth and glistening with an entire translucent edge. The other colony type is flat, mucoid, translucent, grayish and has an irregular edge.

A small percentage of strains may appear tan or slightly pinkish.[11] Colonies tend to spread, especially when initially isolated from fresh clinical specimens.

Limitations of the Procedure

1. Due to the presence of 15 mg/L of cephalothin, growth of *C. fetus* subsp. *fetus* will be inhibited on Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood; therefore, this medium is not recommended for the isolation or culture of this subspecies.
2. Since *C. jejuni* is thermophilic, it is important to incubate the plates at 42°C; otherwise, growth will be delayed. Also, the higher temperature improves selectivity by inhibiting the normal flora.

References

1. Dekeyser, Gossuin-Detrain, Butzler and Sternon. 1972. J. Infect. Dis. *125*:390.
2. Skirrow. 1977. Br. Med. J. *2*:9.
3. Blaser, Cravens, Powers and Wang. 1978. Lancet *ii*:979.
4. Blaser, Berkowitz, LaForce, Cravens, Reller and Wang. 1979. Ann. Intern. Med. *91*:179.
5. Wilson and Wang. October 13, 1979. Background and culture techniques for *Campylobacter fetus* subsp. *jejuni*. Information flier, Campylobacter Laboratory, Veterans Administration Hospital, Denver, Co.
6. Reller, Mirrett and Reimer. 1983. Abstr. C274. Abstr. Annu. Meet. Am. Soc. Microbiol. 1983.
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8. Karmali, Simor, Roscoe, Fleming, Smith and Lane. 1986. J. Clin. Microbiol. *23*:456.
9. Grasmick. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
10. Karmali and Fleming. 1979. J. Clin. Microbiol. *10*:245.
11. Kaplan. 1980. *In* Lennette, Balows, Hausler and Truant (ed.). 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Campylobacter Agar Base

BS12 CMPH2 COMPF ISO MCM9 SMD SMWW

Cat. No.	214892	Dehydrated – 500 g
	218201	Dehydrated – 2 kg

Difco™ Campylobacter Antimicrobic Supplement Skirrow

ISO SMWW

Cat. No.	214891	Vial – 6 × 5 mL

Difco™ Campylobacter Antimicrobic Supplement Blaser

SMWW

Cat. No.	214890	Vial – 6 × 5 mL

BBL™ Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood (Blaser)

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	221727	Prepared Plates – Pkg. of 20*
	221728	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254001	Prepared Plates – Pkg. of 20*
	254069	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251727	Prepared Plates – Pkg. of 20*

BBL™ Campy CSM Agar

BS12 CMPH2 MCM9 SMWW

Cat. No.	299614	Prepared Plates – Pkg. of 20*

BBL™ Campy CVA Agar

BS12 COMPF MCM9

Cat. No.	297246	Prepared Plates – Pkg. of 20*
	297713	Prepared Plates – Ctn. of 100*

BBL™ Skirrows Medium

ISO SMWW

United States and Canada

Cat. No.	297793	Prepared Plates – Pkg. of 20*

Japan

Cat. No.	252111	Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Campylobacter Thioglycollate Medium with 5 Antimicrobics

Intended Use

Campylobacter Thioglycollate Medium with 5 Antimicrobics is recommended as a holding medium for samples suspected to contain *Campylobacter jejuni* subsp. *jejuni* when immediate inoculation of Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood cannot be performed.

Summary and Explanation

In 1972, Dekeyser et al. reported the isolation of *C. jejuni* from the feces of patients with diarrhea and acute gastroenteritis using a filtration technique and a selective medium with antimicrobics to suppress the normal enteric flora.[1] Skirrow, in 1977, reported a selective culture medium containing three antimicrobics.[2] Blaser et al. reported success in isolating *C. jejuni* by direct inoculation of stool specimens onto an agar medium containing four antimicrobics and by inoculating this medium with stool swabs held refrigerated for 8 hours in thioglycollate broth (0.16% agar) containing the same four antimicrobics.[3,4] A fifth antimicrobic, cephalothin, was later incorporated to inhibit nonpathogenic *C. fetus* subsp. *fetus*.[4] The combined yield using Campylobacter blood agar and Campylobacter Thioglycollate Medium, both containing five antimicrobics, was reported to be 33% higher than when the plated medium only was used and 28% higher than when the

broth medium was used alone.[4] Luechterfeld et al. reported that the number of positives was not substantially increased by holding turkey fecal specimens at 4ºC overnight in Campylobacter Thioglycollate Medium.[5]

Campylobacter Thioglycollate Medium has been recommended as a holding medium when facilities for streaking and incubation are not immediately available.[6]

Principles of the Procedure

Campylobacter Thioglycollate Medium is a selective holding medium recommended for the isolation of *C. jejuni* from clinical specimens. The incorporation of antimicrobial agents (i.e., amphotericin B, cephalothin, polymyxin B, trimethoprim and vancomycin) and refrigeration inhibits further multiplication of normal microbial flora in fecal specimens, thus facilitating isolation of *C. jejuni*.

Procedure

1. Sample collection, storage and subculturing to plated medium.[7]
   Place rectal swab about 1 cm into the medium and twirl the swab. Remove the swab or lower it to bottom of the tube and break the shaft of the swab with the lip of the tube to allow easy access to the shaft.
   With solid stools, prepare a saline suspension, blend in a mechanical mixer (i.e., vortex) and place five drops into the medium about 1 cm below the surface. Alternatively, probe all areas of the stool with a swab and inoculate the medium as described for a rectal swab. With diarrheal stools, place five drops in the medium about 1 cm below the surface.
   Refrigerate inoculated Campylobacter Thioglycollate Medium overnight and subculture the next day to Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood plates using a Pasteur pipette inserted about 2 cm below the surface of the broth to continuously withdraw a sample as the tip is slowly drawn to the surface. Do not subculture onto nonselective media since the normal flora may still be viable.
2. Incubation of plated medium.
   Incubate plated medium at 42ºC in a reduced oxygen, increased carbon dioxide atmosphere. This atmosphere can be achieved by using one **BBL™ CampyPak™** Plus disposable gas generator envelope in a **GasPak™** 100 jar, three envelopes in a **GasPak™** 150 jar or using the **Bio-Bag™** Type Cfj or **GasPak** EZ Campy systems. Alternatively, the atmosphere can be achieved using evacuation of **GasPak** vented jars and replacement with cylinder gases,[8] or by using the Fortner principle.[9]

Expected Results

Plates of Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood inoculated from Campylobacter Thioglycollate Medium with 5 Antimicrobics should be examined for the presence of colonies of *Campylobacter jejuni*. These colonies will appear as small, mucoid, usually grayish in coloration, flat with irregular edges and nonhemolytic at 24 and 48 hours.[10]

Colonies may be only barely visible at 18 and 24 hours. An alternate colonial morphology, which appears to be strain related, consists of round colonies 1-2 mm in diameter, which are convex, entire and glistening.[10] A small percentage of strains may appear tan or slightly pinkish in coloration.[7]

Colonies tend to spread or swarm, especially when initially isolated from fresh clinical specimens.

NOTE: If plates are examined after 24 hours of incubation, treat plates as if they were anaerobic cultures; i.e., examine plates quickly and place them back into a reduced oxygen atmosphere immediately after examination.

References

1. Dekeyser, Gossuin-Detrain, Butzler and Sternon. 1972. J. Infect. Dis. *125*:390
2. Skirrow. 1977. Br. Med. J. *2*:9.
3. Blaser, Cravens, Powers and Wang. 1978. Lancet *2*:979
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7. Kaplan. 1980. *In*. Lennette, Balows, Hausler and Truant (ed.), Manual of Clinical Microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
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9. Karmali and Fleming. 1979. J. Clin. Microbiol. *10*:245.
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Availability

BBL™ Campylobacter Thioglycollate Medium with 5 Antimicrobics

Cat. No.	221747	Prepared Tubes – Pkg. of 10
	221748	Prepared Tubes – Ctn. of 100

Candida BCG Agar Base
Candida Bromcresol Green Agar

Intended Use

Candida Bromcresol Green (BCG) Agar is a differential and selective medium used for primary isolation and detection of *Candida* species from clinical specimens.

Summary and Explanation

Candida BCG medium employs the formula devised by Harold and Snyder.[1] They demonstrated that the triphenyltetrazolium chloride (TTC) being used as an indicator in Pagano Levin

User Quality Control

Identity Specifications

Difco™ Candida BCG Agar Base

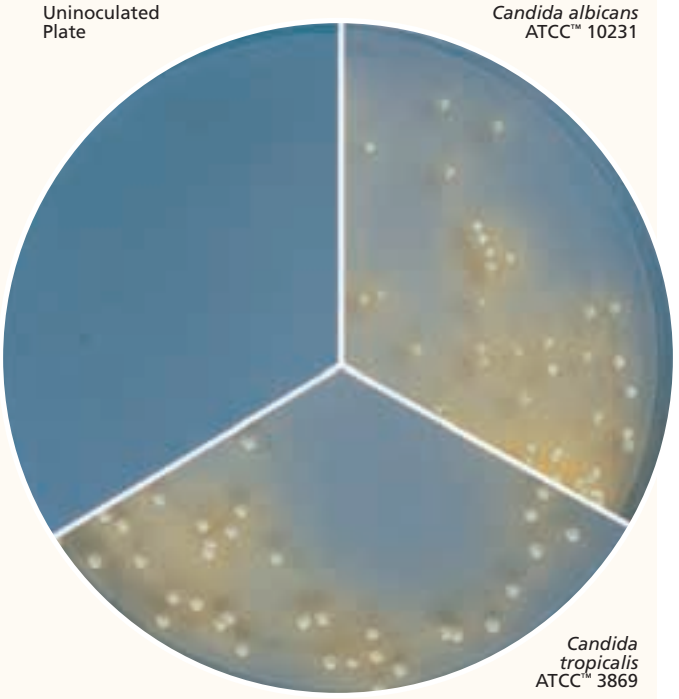
Dehydrated Appearance:	Beige to blue-green, free-flowing, homogeneous.
Solution:	6.6% solution, soluble in purified water upon boiling. Solution is blue-green to green-blue, slightly opalescent to opalescent, may have a precipitate.
Prepared Appearance:	Blue-green to green-blue, slightly opalescent to opalescent.
Reaction of 6.6% Solution at 25°C:	pH 6.1 ± 0.1

Cultural Response

Difco™ Candida BCG Agar Base

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 24-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF MEDIUM
Candida albicans	10231	10^2-10^3	Good	Yellow
Candida tropicalis	9968	10^2-10^3	Good	Yellow
Escherichia coli	25922	10^3	Inhibition	Green



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medium retarded the growth of some species of *Candida* and completely inhibited the growth of others. To overcome this, they replaced TTC with bromcresol green, a non-toxic indicator, to develop Candida BCG Agar. Neomycin is incorporated to inhibit gram-negative and some gram-positive bacteria.

Principles of the Procedure

This medium consists of peptone agar base supplemented with yeast extract and dextrose to provide the nutrients necessary to support growth. Neomycin is an aminoglycoside antibiotic that is active against aerobic and facultatively anaerobic gram-negative bacteria and certain gram-positive species. Bromcresol green aids in differentiation and identification of *Candida* species based on dextrose fermentation. A change in the pH causes the medium to become a yellow color around the colonies of organisms that ferment dextrose.

Formula

Difco™ Candida BCG Agar Base

Approximate Formula* Per Liter

Peptone	10.0	g
Yeast Extract	1.0	g
Dextrose	40.0	g
Agar	15.0	g
Bromcresol Green	0.02	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 66 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Add sterile neomycin (500 µg/mL) to the medium at 50-55°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 30 ± 2°C for up to 72 hours.

Expected Results

Candida species produce convex to cone-shaped, smooth to rough colonies. The color of the medium around the colonies becomes yellow, usually within 72 hours.

Gram staining, biochemical tests and serological procedures should be performed to confirm findings.[2-4]

References

1. Harold and Snyder. 1968. Personal communication.
2. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
3. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Candida BCG Agar Base

Cat. No. 283510 Dehydrated – 500 g

BBL™ Candida Bromcresol Green Agar

Cat. No. 296241 Prepared Plates (complete) – Pkg. of 20*

**Store at 2-8°C.*

Cary and Blair Transport Medium

(See Transport Media)

Casamino Acids
Bacto™ Casamino Acids • Bacto™ Casamino Acids, Technical • Casamino Acids, Vitamin Assay Acidicase™ Peptone

Intended Use

Bacto Casamino Acids and **Bacto** Casamino Acids, Technical are used in preparing microbiological culture media.

Casamino Acids, Vitamin Assay is used in vitamin assay procedures.

Acidicase Peptone is used as a nutritional supplement in vitamin assay, susceptibility testing and other laboratory media and microbial fermentation where the high salt content will not interfere.

Summary and Explanation

Bacto Casamino Acids is an acid hydrolysate of casein, prepared according to the method described by Mueller and Miller.[1] The method described reduces the sodium chloride and iron content of the hydrolyzed casein. This hydrolyzed casein, supplemented with inorganic salts, growth factors, cystine, maltose and an optimum amount of iron was used by Mueller and Miller to prepare diphtheria toxin. **Bacto** Casamino Acids duplicates this specially treated hydrolyzed casein.

Bacto Casamino Acids, due to the nearly complete hydrolysis of casein and the low sodium chloride and iron content, makes an excellent supplement for many media formulations where nitrogen requirements are minimal. This product has been recommended as a compromise for the replacement of pure amino acids in a defined medium for the growth of *Lactobacillus*, thus eliminating the complexity of preparation.[2] Additionally, it has been successfully used, along with Tryptone Peptone, in nutritional studies to determine a bacterium's growth requirement for peptides or amino acids.[3,4] It also works well as a component in laboratory media. It has been utilized in such diverse applications as TYI-S-33 media for the parasite *Entamoeba histolytica* and LCM medium for the growth of a nematode-bacterium complex.[5]

Bacto Casamino Acids, Technical is an acid hydrolysate of casein. The hydrolysis is carried out as in the preparation of **Bacto** Casamino Acids, but the sodium chloride and iron content of this product have not been decreased to the same extent. **Bacto** Casamino Acids, Technical is recommended for use in culture media where amino acid mixtures are required for a nitrogen source and the sodium chloride content is slightly increased. It is particularly valuable in studying the growth requirements of bacteria.

Bacto Casamino Acids, Technical is prepared according to the method suggested by Mueller[1] for use in the preparation of diphtheria toxin. **Bacto** Casamino Acids, Technical has been used in a medium for primary isolation of gonococcus and meningococcus, in agar-free media for the isolation of *Neisseria*, in a tellurite medium for the isolation of *Corynebacterium* and in the preparation of a medium for the testing of disinfectants.[6-8]

Casamino Acids, Vitamin Assay is an acid digest of casein specially treated to markedly reduce or eliminate certain vitamins. It is recommended for use in microbiological assay media and in studies of the growth requirements of microorganisms. Casamino Acids, Vitamin Assay is commonly used as the amino acid source in early phases of nutrition work.[9] Casamino Acids, Vitamin Assay was used as the acid hydrolyzed casein in studies on *p*-aminobenzoic acid and *p*-teroylglutamic acid as growth factors for *Lactobacillus* species.[10]

Several media containing Casamino Acids are specified in standard methods for multiple applications.[11-16]

Acidicase Peptone is a hydrochloric acid hydrolysate of casein. The manufacturing process produces a casein hydrolysate that has a high salt content of approximately 37% and nitrogen content of approximately 8%. The hydrolysis of the casein, a milk protein rich in amino acid nitrogen, is carried out until all the nitrogen is converted to amino acids or other compounds of relative simplicity. It is deficient in cystine, because casein contains little cystine, and in tryptophan, which is destroyed by the acid treatment.

Principles of the Procedure

Bacto Casamino Acids, **Bacto** Casamino Acids, Technical, Casamino Acids, Vitamin Assay and **Acidicase** Peptone are acid hydrolyzed casein. Casein is milk protein and a rich source of amino acid nitrogen. **Bacto** Casamino Acids, **Bacto** Casamino Acids, Technical, Casamino Acids, Vitamin Assay and **Acidicase** Peptone provide nitrogen, vitamins, carbon and amino acids in microbiological culture media. Although **Bacto** Casamino Acids, **Bacto** Casamino Acids, Technical, Casamino Acids, Vitamin Assay and **Acidicase** Peptone are added to media primarily because of their organic nitrogen and growth factor components, their inorganic components also play a vital role.[17]

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Casamino Acids

Dehydrated Appearance: Very light beige, free-flowing, homogeneous.

Solution: 1.0% solution, soluble in purified water upon slight heating. Solution is very light amber, clear. 2.0% solution, soluble in purified water upon slight heating. Solution is light amber, clear.

Reaction of 2.0% Solution at 25°C: pH 5.8-6.65

Bacto™ Casamino Acids, Technical

Dehydrated Appearance: Very light beige, free-flowing, homogeneous.

Solution: 1.0% solution, soluble in purified water. Solution is colorless to very light amber, clear.

Reaction of 1.0% Solution at 25°C: pH 5.0-7.5

Difco™ Casamino Acids, Vitamin Assay

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 3.0% solution, soluble in purified water upon boiling. Solution is very light to light amber, clear, may have a slight precipitate.

Reaction of 3.0% Solution at 25°C: pH 6.5-8.5

BBL™ Acidicase™ Peptone

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 2.0% solution, soluble in purified water. Solution is clear to slightly hazy.

Reaction of 2.0% Solution at 25°C: pH 6.5-7.5

Cultural Response

Bacto™ Casamino Acids or Bacto™ Casamino Acids, Technical

Prepare a sterile 1% solution and adjust the pH to 7.2 ± 0.2. Inoculate and incubate tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhi	19430	10^2-10^3	Good

Difco™ Casamino Acids, Vitamin Assay

Prepare various vitamin assay media using Casamino Acids, Vitamin Assay to determine the vitamin content. It should not contain a vitamin content higher than 20% above the following values:

Vitamin B_{12}	0.2	ng/g
Biotin	0.3	ng/g
Folic Acid	3.3	ng/g
Niacin	0.17	µg/g
Pantothenate	0.04	µg/g
Riboflavin	0.1	µg/g
Thiamine	0.1	µg/g

BBL™ Acidicase™ Peptone

Prepare a sterile solution of 10.0 g of **Acidicase** Peptone, 2.5 g of sodium chloride and 6.5 g of agar in 500 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 2-3 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-10^4	Good
Pseudomonas aeruginosa	27853	10^3-10^4	Good
Staphylococcus aureus	25923	10^3-10^4	Good

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** Casamino Acids, **Bacto** Casamino Acids, Technical, Casamino Acids, Vitamin Assay and **Acidicase** Peptone in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for specific procedures using these hydrolysates.

Expected Results

Refer to appropriate references and procedures for results.

References

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12. Downs and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
13. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
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15. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.
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Availability

Bacto™ Casamino Acids

AOAC BAM COMPF SMWW USDA USP

Cat. No.	223050	Dehydrated – 500 g
	223020	Dehydrated – 2 kg
	223030	Dehydrated – 10 kg

Bacto™ Casamino Acids, Technical

Cat. No.	223120	Dehydrated – 500 g
	223110	Dehydrated – 10 kg

Difco™ Casamino Acids, Vitamin Assay

Cat. No.	228820	Dehydrated – 100 g
	228830	Dehydrated – 500 g

BBL™ Acidicase Peptone

AOAC BAM COMPF SMWW USDA USP

Cat. No.	211843	Dehydrated – 500 g

Casein Agar

(See Nocardia Differentiation Media)

Casein Digest

Intended Use

Casein Digest is used in preparing microbiological culture media.

Summary and Explanation

Casein Digest, an enzymatic digest of casein similar to N-Z-Amine A, was developed for use in molecular genetics media.

User Quality Control

Identity Specifications

Difco™ Casein Digest

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1%, 2%, and 10% solutions, soluble in purified water. Solutions are: 1%-Light to medium amber, clear; 2%-Medium amber, clear; 10%-Dark amber, clear.
Reaction of 1% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Casein Digest

Prepare NZM Broth per formula. Inoculate and incubate at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10^2-10^3	Good
Escherichia coli (HB101)	33694	10^2-10^3	Good
Escherichia coli (JM107)	47014	10^2-10^3	Good
Escherichia coli (DH5)	53868	10^2-10^3	Good
*Saccharomyces cerevisiae**	9763	10^2-10^3	Good
Streptomyces avermitilis	31267	10^2-10^3	Fair to good

**Tested with addition of 0.5% dextrose.*

This product is digested under conditions different from other enzymatic digests of casein, including Tryptone and Casitone.

Casein Digest is contained in the formulas of NZ media (NZCYM Broth, NZYM Broth and NZM Broth), which are used for cultivating recombinant strains of *Escherichia coli*. *E. coli* grows rapidly in these rich media because they provide amino acids, nucleotide precursors, vitamins and other metabolites that the cells would otherwise have to synthesize.[1] Consult appropriate references for recommended test procedures using NZ media.[1,2]

Principles of the Procedure

Casein Digest is a nitrogen and amino acid source for microbiological culture media. Casein is raw milk protein, a rich source of amino acid nitrogen.

Procedure

See appropriate references for specific procedures using Casein Digest.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols in molecular biology, vol.1. Current Protocols, New York, N.Y.
2. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Availability

Difco™ Casein Digest

Cat. No. 211610 Dehydrated – 500 g

Bacto™ Casitone • Trypticase™ Peptone Bacto™ Tryptone • BiTek™ Tryptone

Intended Use

Bacto Casitone, **Trypticase** Peptone, **Bacto** Tryptone and **BiTek** Tryptone are used in preparing microbiological culture media.

Ingredients, where noted, meet *United States Pharmacopeia (USP) performance specifications.*

Summary and Explanation

The manufacturing process for an enzymatic digest of casein is not as destructive as an acid hydrolysis. Thus, the casein is not broken down as completely into its constituent components. In many cases this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids.

Bacto Casitone can be used as a component in microbiological culture media or in fermentation applications. A recent publication has also reported that the stability of lyophilized influenza virus vaccine was augmented by the addition of 2% Casitone.[1]

Trypticase Peptone is the primary nitrogen source in **Trypticase** Soy Broth and Agar. This product is recommended for use in media formulations, where good growth of fungi and bacteria is required. **Trypticase** Peptone is referenced in *Official Methods*

of Analysis of AOAC International and meets specifications in the *USP* for pancreatic digest of casein.[2,3]

Bacto Tryptone was developed by Difco Laboratories while investigating a peptone particularly suitable for the elaboration of indole by bacteria. It is also notable for the absence of detectable levels of carbohydrates. **Bacto** Tryptone has been used in conjunction with casamino acids in nutritional studies to determine amino acids vs. peptide utilization.[4,5] It is included in standard methods applications and is listed in the reagent section of the *USP* as meeting the specifications for pancreatic digest of casein, a component in many of the media listed.[2,3,6-11] The *European Pharmacopoeia* also lists pancreatic digest of casein as a component in many of the recommended media.[12] **Bacto** Tryptone also works well in fermentation applications. It has been used successfully with commonly used organisms, such as *Escherichia coli*,[13] as well as uncommon organisms, such as the diatom *Nitzschia laevis*.[14]

BiTek Tryptone is prepared similarly to **Bacto** Tryptone but the final product goes through fewer refinement steps during processing. This product provides some of the same benefits as **Bacto** Tryptone in instances where a less refined hydrolysate can be utilized.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Casitone

Dehydrated Appearance:	Tan, free-flowing, granules.
Solution:	1.0%, 2.0% and 10.0 % solutions, soluble in purified water. 1.0% solution is light amber, clear. 2.0% solution is light to medium amber, clear, may have a slight precipitate. 10.0% solution is medium to dark amber, clear to very slightly opalescent, may have a precipitate.
Reaction of 1.0% Solution at 25°C:	pH 6.8-7.4

Bacto™ Tryptone

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light to light amber, clear. 2.0% solution is light to medium amber, clear. 10.0% solution is medium to dark amber, clear to slightly opalescent,may have a slight precipitate.
Reaction of 2.0% Solution at 25°C:	pH 6.5-7.5

BiTek™ Tryptone

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light to light amber, clear. 2.0% solution is light to medium amber, clear. 10.0% solution is medium to dark amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 2.0% Solution at 25°C:	pH 7.2 ± 0.2

BBL™ Trypticase™ Peptone

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water. Solution is clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 6.5-7.5

Continued

Principles of the Procedure

Bacto Casitone, **Trypticase** Peptone, **Bacto** Tryptone and **BiTek** Tryptone are pancreatic digests of casein. Casein is the main milk protein and a rich source of amino acid nitrogen.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** Casitone, **Trypticase** Peptone, **Bacto** Tryptone and **BiTek** Tryptone in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for specific procedures using **Bacto** Casitone, **Trypticase** Peptone, **Bacto** Tryptone and **BiTek** Tryptone.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Yannarell, Goldberg and Hjorth. 2001. J. Virol. Methods (in press).
2. Horowitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
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4. Takahashi and Yamada. 2000. J. Bacteriol. *182*:4704.
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8. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington D.C.
9. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington D.C.
10. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, Washington, D.C.
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12. Council of Europe. 2008. European pharmacopoeia, 6th ed. Council of Europe, Strasbourg, France.
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14. Wen and Chen. 2001. Enzyme Microbia Technol. 29:341.

Cultural Response

Biochemical Reactions

Bacto™ Casitone, Bacto™ Tryptone or BiTek™ Tryptone

Prepare a sterile solution as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922	~10^7	Negative
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	0.1 mL, undiluted	Positive

BBL™ Trypticase™ Peptone

Prepare a sterile solution as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	29552	~10^7	Negative
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Citrobacter freundii*	8454	0.1 mL, undiluted	Positive

Growth Response

Bacto™ Casitone, Bacto™ Tryptone or BiTek™ Tryptone

Prepare a sterile solution with 2.0% **Bacto** Casitone, **Bacto** Tryptone or **BiTek** Tryptone, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	30-300	Good
Staphylococcus aureus	25923	30-300	Good

BBL™ Trypticase™ Peptone

1. Prepare a sterile solution of peptone agar without (plain) and with 5% sheep blood (SB) using 10 g Trypticase Peptone, 2.5 g sodium chloride and 6.5 g agar in 500 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 3 days (incubate streptococci with CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Enterobacter aerogenes	13048	10^3-10^4	Good	N/A
Escherichia coli	25922	10^3-10^4	Good	N/A
Staphylococcus aureus	6538P	10^3-10^4	Good	N/A
Staphylococcus epidermidis	12228	10^3-10^4	Good	N/A
Streptococcus agalactiae	12386	10^3-10^4	N/A	Good, beta hemolysis
Streptococcus pneumoniae	6305	10^3-10^4	N/A	Good, alpha hemolysis
Streptococcus pyogenes	49117	10^4-10^5	Good	Good, beta hemolysis

2. Prepare a sterile solution of chocolate peptone agar using **Trypticase** Peptone. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 3 days with CO_2.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria gonorrhoeae	19424	10^3-10^4	Good

Availability

Bacto™ Casitone

COMPF SMD SMWW USDA

Cat. No. 225930 Dehydrated – 500 g
225910 Dehydrated – 10 kg

BBL™ Trypticase™ Peptone

AOAC BAM COMPF EP EPA SMD SMWW USDA USP

Cat. No. 211921 Dehydrated – 454 g
211922 Dehydrated – 5 lb (2.3 kg)
211923 Dehydrated – 25 lb (11.3 kg)

Bacto™ Tryptone

AOAC BAM COMPF EP EPA SMD SMWW USDA USP

Cat. No. 211705 Dehydrated – 500 g
211699 Dehydrated – 2 kg
211701 Dehydrated – 10 kg

BiTek™ Tryptone

Cat. No. 251420 Dehydrated – 10 kg

Casman Agar Base

Intended Use

Casman Agar Base is used for the cultivation of fastidious pathogenic organisms, such as *Haemophilus influenzae* and *Neisseria gonorrhoeae*, from clinical specimens.

Summary and Explanation

Members of the genus *Haemophilus* are fastidious microorganisms that require the addition of X and/or V growth factors for *in vitro* cultivation.[1] *Neisseria* are also fastidious microorganisms with complex growth requirements.[2]

In 1947, Casman described a blood-enriched medium prepared without an infusion of fresh meat for cultivation of *Haemophilus* and gonococci.[1] The medium was developed to replace previous formulations that required time-consuming preparations of fresh and heated blood and fresh meat infusion to supply the nutrients necessary for the growth of these fastidious organisms.[2,3]

Casman found that nicotinamide interfered with the activity of an enzyme in blood that inactivates V factor (NAD). Using unheated human blood, he found that amount of nicotinamide required for good growth of *H. influenzae* was inhibitory to gonococci.[2] Therefore, he reduced the nicotinamide to a level that allowed good growth of gonococci.

User Quality Control

Identity Specifications

BBL™ Casman Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.3% solution, soluble in purified water upon boiling. Solution is medium to dark, yellow to tan, hazy to cloudy, with a moderate to large amount of cream flocculation.
Prepared Appearance:	Medium to dark, yellow to tan, hazy to cloudy, with a moderate to large amount of cream flocculation.
Reaction of 4.3% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Casman Agar Base

Prepare the medium per label directions. Inoculate and incubate for 42-48 hours at 35 ± 2°C, aerobically for *L. monocytogenes* and with 3-5% CO_2 for all other organisms.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
Haemophilus influenzae	10211	10^2-10^3	Good	N/A
Haemophilus parahaemolyticus	10014	10^2-10^3	Good	Beta
Listeria monocytogenes	19115	10^2-10^3	Good	Weak beta
Neisseria gonorrhoeae	43070	10^2-10^3	Good	N/A
Streptococcus pyogenes	19615	10^2-10^3	Good	Beta

To improve the recovery of *H. influenzae* on this medium, horse or rabbit blood should be used instead of human blood, since they contain less NADase.[4]

Principles of the Procedure

Casman Agar Base is a nonselective, peptone-based medium. The peptones and beef extract provide amino acids and other complex nitrogenous nutrients. Yeast extract is a source of the B-complex vitamins.

Supplementing Casman Agar Base with blood supplies the growth factors required by *H. influenzae* – hemin, or X factor, and nicotinamide adenine dinucleotide (NAD), or V factor. Horse and rabbit bloods are preferred by some laboratories because they are relatively free of NADase, an enzyme that destroys the V factor. The addition of lysed blood stimulates the growth of some strains of *N. gonorrhoeae*. Nicotinamide is incorporated into the medium to inhibit the nucleotidase of erythrocytes that destroys the V factor.

Cornstarch is incorporated to prevent fatty acids from inhibiting the growth of *N. gonorrhoeae* and to facilitate β-hemolytic reactions by neutralizing the inhibitory action of dextrose. A small amount of dextrose is added to enhance the growth of pathogenic cocci.

Formula

BBL™ Casman Agar Base

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	11.5	g
Peptic Digest of Animal Tissue	5.0	g
Yeast Extract	3.5	g
Beef Extract	3.0	g
Nicotinamide	0.05	g
p-Aminobenzoic Acid	0.05	g
Dextrose	0.5	g
Cornstarch	1.0	g
Sodium Chloride	5.0	g
Agar	13.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 43 g of powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C and add 5% sterile blood and 0.15% blood solution, made by lysing 1 part of blood with 3 parts of water. Alternatively, add 5% partially lysed blood.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the isolation and identification of *Neisseria* and *Haemophilus*, consult appropriate references.[5,6]

Expected Results

H. influenzae produces colorless to gray, transparent, moist colonies with a characteristic "mousy" odor. *N. gonorrhoeae* produces small, translucent, raised, moist, colorless to grayish-white colonies.

Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

References

1. Casman. 1947. Am. J. Clin. Pathol. *17*:281.
2. Casman. 1942. J. Bacteriol. *43*:33.
3. Casman. 1947. J. Bacteriol. *53*:561.
4. Krumweide and Kuttner. 1938. J. Exp. Med. *67*:429.
5. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Casman Agar Base

Cat. No. 211106 Dehydrated – 500 g

Cetrimide Agar Base • Pseudosel™ Agar

Intended Use

Cetrimide (**Pseudosel**) Agar is used for the selective isolation and identification of *Pseudomonas aeruginosa*.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

King et al. developed Medium A (Tech Agar) for the enhancement of pyocyanin production by *Pseudomonas*.[4] Cetrimide (**Pseudosel**) Agar has the formula for Tech Agar but is modified by the addition of cetrimide (cetyl trimethyl ammonium bromide) for the selective inhibition of organisms other than *P. aeruginosa*.[5]

In 1951, Lowbury described the use of 0.1% cetrimide in a selective medium for *P. aeruginosa*.[5] Because of the increased purity of the inhibitory agent, the concentration was later reduced, as reported by Lowbury and Collins in 1955.[6] Brown and Lowbury employed incubation at 37°C with examination after 18 and 42 hours of incubation.[7]

Strains of *P. aeruginosa* are identified from specimens by their production of pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment in addition to their colonial morphology[8] and the characteristic grapelike odor of aminoacetophenone.[9] *P. aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. Cetrimide (**Pseudosel**) Agar, therefore, is a valuable culture medium in the identification of this organism.

Cetrimide (**Pseudosel**) Agar is widely recommended for use in the examination of cosmetics,[10] clinical specimens[8,11] for the presence of *P. aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism.[12] It is also used in the microbiological examination of nonsterile pharmaceutical products for *Pseudomonas aeruginosa*.[1]

Principles of the Procedure

Gelatin peptone supplies the nutrients necessary to support growth. The production of pyocyanin is stimulated by the magnesium chloride and potassium sulfate in the medium.[13] Cetrimide is a quaternary ammonium, cationic detergent compound, which is inhibitory to a wide variety of bacterial species including *Pseudomonas* species other than *P. aeruginosa*. Agar is a solidifying agent. Cetrimide Agar Base is supplemented with 1% glycerol as a source of carbon.

Formula

Difco™ Cetrimide Agar Base

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	20.0	g
Magnesium Chloride	1.4	g
Potassium Sulfate	10.0	g
Cetrimide (Tetradecyltrimethylammonium Bromide)	0.3	g
Agar	13.6	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45.3 g of the powder in 1 L of purified water containing 10 mL of glycerol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates in an inverted position (agar side up) at 35 ± 2°C for 18-48 hours.

Inoculate tubes with either pure cultures or with specimen material. Incubate tubes at 35 ± 2°C for 18-24 hours in an aerobic atmosphere.

Refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products and tests for isolating *Pseudomonas aeruginosa* using Cetrimide Agar.[1]

Expected Results

Colonies that are surrounded by a blue-green pigment and fluoresce under short wavelength (254 nm) ultraviolet light may be presumptively identified as *Pseudomonas aeruginosa*. Note, however, that certain strains of *P. aeruginosa* may not produce pyocyanin. Other species of *Pseudomonas* do not produce pyocyanin, but fluoresce under UV light. Most non-*Pseudomonas*

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Cetrimide Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.53% solution with 1% glycerol, soluble in purified water upon boiling. Solution is light amber, opalescent, with a precipitate.
Prepared Appearance:	Light amber, opalescent, with precipitate.
Reaction of 4.53% Solution with 1% glycerol at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Cetrimide Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. Incubate plates with *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027 at 30-35°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	10^3- 2 × 10^3	Inhibition	—
Pseudomonas aeruginosa	27853	10^3	Good	Yellow-green to blue
Staphylococcus aureus	25923	10^3- 2 × 10^3	Inhibition	—
Pseudomonas aeruginosa	9027	10-100	Growth	N/A
Escherichia coli	8739	>100	No growth	N/A

Identity Specifications

BBL™ Pseudosel™ Agar (prepared)

Appearance:	Cream to cream-tan and trace hazy to hazy.
Reaction at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ Pseudosel™ Agar (prepared)

Inoculate and incubate at 35-37°C for 18–48 hours. Incubate plates with *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027 at 30-35°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-10^4	Inhibition
Pseudomonas aeruginosa	10145	10^3-10^4	Good
Stenotrophomonas maltophilia	13637	10^3-10^4	Inhibition
Pseudomonas aeruginosa	9027	10-100	Growth
Escherichia coli	8739	>100	No growth

Pseudosel™ Agar

species are inhibited, and some species of *Pseudomonas* may also be inhibited. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

Limitations of the Procedure

1. The type of peptone used in the base may affect pigment production.[7,14]
2. No single medium can be depended upon to exhibit all pigment-producing *P. aeruginosa* strains.
3. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescin production since this yellowing does not fluoresce.[7]
4. Some nonfermenters and some aerobic sporeformers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.[7]
5. Studies of Lowbury and Collins[6] showed *P. aeruginosa* may lose its fluorescence under UV light if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed. online. Japanese Ministry of Health, Labour and Welfare.
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8. Blondel-Hill, Henry and Speert. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (eds.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
9. Gilardi. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (eds.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
10. Hitchins, Tran, and McCarron. 2001. *In* FDA bacteriological analytical manual online, 8th ed. http://www.cfsan.fda.gov/~ebam/bam-23.html.
11. Forbes, Sahm, and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby Elsevier, St. Louis, Mo.
12. Horwitz, (ed). 2002. AOAC Official Method 955.13. Official methods of analysis of AOAC International, 17th ed, vol. 1, Rev. 1. AOAC International, Gaithersburg, Md.
13. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
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Availability

Difco™ Cetrimide Agar Base

AOAC BAM BS12 CCAM EP JP MCM9 USP

Cat. No. 285420 Dehydrated – 500 g†

BBL™ Pseudosel™ Agar

AOAC BAM BS12 CCAM EP JP MCM9 USP

United States and Canada

Cat. No. 297882 Prepared Plates – Pkg. of 10*†
221344 Tubed Slants – Pkg. of 10
221345 Tubed Slants – Ctn. of 100

Europe

Cat. No. 254419 Prepared Plates – Pkg. of 20*†

Mexico (Cetrimide Agar)

Cat. No. 252626 Prepared Plates (60 × 15 mm-style) – Pkg. of 10*†
257506 Prepared Plates – Pkg. of 10*†

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

** Store at 2-8°C.*
† QC testing performed according to USP/EP/JP performance specifications.

Chapman Stone Medium

Intended Use

Chapman Stone Medium is used for isolating and differentiating staphylococci based on mannitol fermentation and gelatinase activity.

Summary and Explanation

Chapman Stone Medium is prepared according to the formula described by Chapman.[1] It is similar to Staphylococcus Medium 110, previously described by Chapman,[2] except that the sodium chloride concentration is reduced to 5.5% and ammonium sulfate is included in the formulation. The inclusion of ammonium sulfate in the medium negates the need to add a reagent after growth has been obtained in order to detect gelatinase activity by Stone's method. Chapman Stone Medium is especially recommended for suspected food poisoning studies involving *Staphylococcus*.[3] It is selective, due to the relatively high salt content, and is differential due to pigmentation, mannitol fermentation and the presence or absence of gelatin liquefaction.

Principles of the Procedure

Yeast extract and peptone provide nitrogen, carbon, sulfur, vitamins and trace nutrients essential for growth. Gelatin serves as a substrate for gelatinase activity. Ammonium sulfate allows detection of gelatin hydrolysis. D-Mannitol is the fermentable carbohydrate. Sodium chloride acts as a selective agent because most bacterial species are inhibited by the high salt content. Dipotassium phosphate provides buffering capability. Agar is the solidifying agent.

Formula

Difco™ Chapman Stone Medium

Approximate Formula* Per Liter		
Yeast Extract	2.5	g
Pancreatic Digest of Casein	10.0	g
Gelatin	30.0	g
D-Mannitol	10.0	g
Sodium Chloride	55.0	g
Ammonium Sulfate	75.0	g
Dipotassium Phosphate	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 20.2 g of the powder in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes. Omit autoclaving if used within 12 hours.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Chapman Stone Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous with a tendency to cake.
Solution:	20.2% solution, soluble in purified water upon boiling. Solution is light amber, opalescent with a precipitate.
Prepared Appearance:	Light to medium amber, opalescent with a precipitate.
Reaction of 20.2% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Chapman Stone Medium

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours. Add bromcresol purple indicator to determine mannitol fermentation (yellow = positive).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HALO (GELATINASE)	MANNITOL FERMENTATION
Escherichia coli	25922	10^2-10^3	Inhibition	–	–
Staphylococcus aureus	25923	10^2-10^3	Good	+	+
Staphylococcus epidermidis	12228	10^2-10^3	Good	+	–

Procedure

1. Streak a sample of the specimen onto the surface of the agar. Make several stabs into the medium along the streak.
2. Incubate, aerobically, at 30 ± 2°C for up to 48 hours.
3. Examine for growth and the presence or absence of clear zones around colonies.
4. To determine mannitol fermentation, add a few drops of bromcresol purple to areas on the medium from which colonies have been removed. Any change in color of the indicator, compared with that of the uninoculated medium, indicates fermentation of mannitol.

Expected Results

Mannitol fermentation: Positive = change in color of the indicator to yellow.

Gelatinase activity: Positive Stone reaction = formation of clear zones around the colonies.

Any mannitol-positive, yellow or orange colonies surrounded by a clear zone are presumptively identified as *Staphylococcus aureus*. White or nonpigmented colonies, with or without a clear zone, are probably *S. epidermidis*.

Limitations of the Procedure

1. Confirm the presumptive identification of pathogenic staphylococci with additional tests, such as coagulase activity.
2. Enterococci and/or Group D streptococci may exhibit growth on the medium and show slight mannitol fermentation. The colonies, however, are tiny and can easily be differentiated from staphylococci by Gram stain and the catalase test.[3]

References

1. Chapman. 1948. Food Res. *13*:100.
2. Chapman. 1946. J. Bacteriol. *51*:409.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Chapman Stone Medium

Cat. No. 211805 Dehydrated – 500 g

C

Charcoal Agar

Intended Use

Charcoal Agar is used for cultivating fastidious organisms, especially *Bordetella pertussis*, for vaccine production and stock culture maintenance.

Summary and Explanation

Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen.[1] The authors found this medium to be an efficient substitute for Bordet-Gengou Agar in the production of *B. pertussis* vaccines.

The genus *Bordetella* consists primarily of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium;* additional species have recently been described.[2] All *Bordetella* are respiratory pathogens, residing on the mucous membranes of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder form of the disease.[3] *B. bronchiseptica* is an opportunistic human pathogen associated with both respiratory and non-respiratory infections, often occurring in patients having close contact with animals.[2] *B. bronchiseptica* has not been reported to cause pertussis. There have been no reports of recovery of *B. avium* from humans.[2]

User Quality Control

Identity Specifications

Difco™ Charcoal Agar

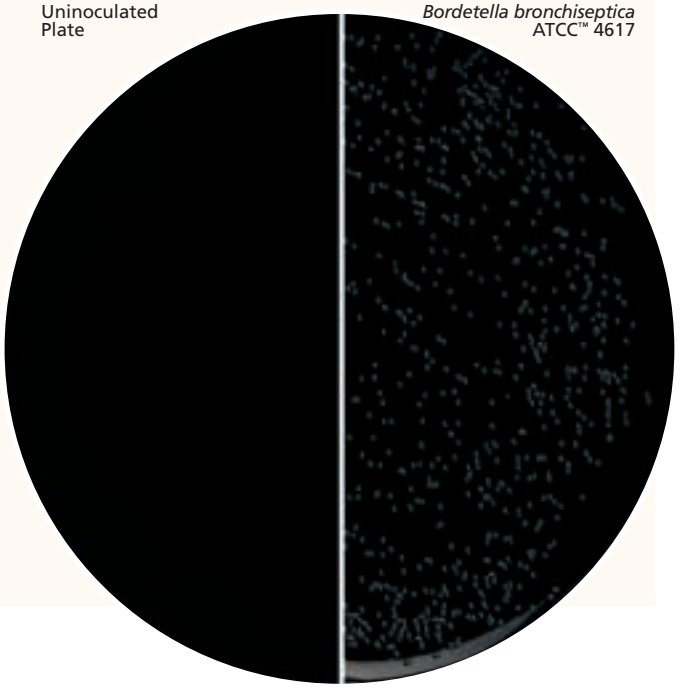
Dehydrated Appearance:	Gray, free-flowing, homogeneous.
Solution:	6.25% solution, soluble in purified water upon boiling. Solution is black, opaque with a precipitate.
Prepared Appearance:	Black, opaque.
Reaction of 6.25% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Charcoal Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bordetella bronchiseptica	4617	10^2-10^3	Good
Bordetella parapertussis	15237	10^2-10^3	Good
Bordetella pertussis	8467	10^2-10^3	Good



Charcoal Agar supplemented with Horse Blood is used for the cultivation and isolation of *Haemophilus influenzae*.[4]

Principles of the Procedure

Infusion from beef heart and peptone provide the nitrogen, carbon and amino acids in Charcoal Agar. Yeast extract is a vitamin source. Sodium chloride maintains osmotic balance. Agar is the solidifying agent. Soluble starch and Norit SG, charcoal, neutralize substances toxic to *Bordetella* species, such as fatty acids.

Formula

Difco™ Charcoal Agar

Approximate Formula* Per Liter		
Beef Heart, Infusion from 500 g	12.0	g
Peptone	10.0	g
Sodium Chloride	5.0	g
Soluble Starch	10.0	g
Yeast Extract	3.5	g
Norit SG	4.0	g
Agar	18.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 62.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Mix thoroughly during dispensing to uniformly distribute the charcoal.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the isolation and maintenance of fastidious microorganisms refer to the procedures described in appropriate references.[2,4,5]

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.[4]

References

1. Mishulow, Sharpe and Cohen. 1953. Am. J. Public Health, *43*:1466.
2. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Linneman and Pery. 1977. Am. J. Dis. Child. *131*:560.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1. Williams & Wilkins, Baltimore, Md.
5. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Charcoal Agar

Cat. No. 289410 Dehydrated – 500 g

Chocolate II Agar • Chocolate II Agar with Bacitracin Chocolate II Agar with Pyridoxal

Intended Use

Chocolate II Agar is an improved medium for use in qualitative procedures for the isolation and cultivation of fastidious microorganisms, especially *Neisseria* and *Haemophilus* species, from a variety of clinical specimens. Media provided in divided (two-sectored) plates offer the ability to utilize the properties of two media in one plate.

Chocolate II Agar with Bacitracin is a selective medium used for the isolation of *Haemophilus* species.

Chocolate II Agar with Pyridoxal is used for the isolation of nutritionally-variant streptococci (vitamin B_6-requiring streptococci) from blood cultures.

Summary and Explanation

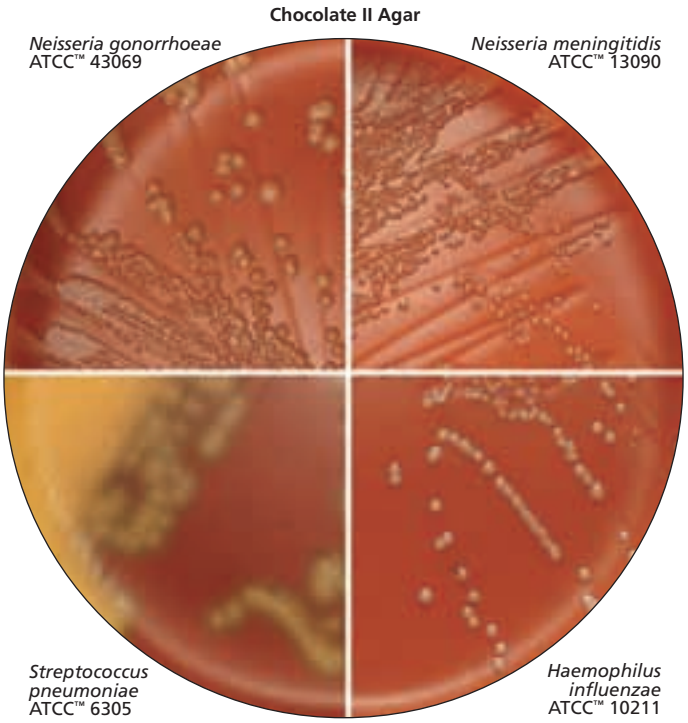
Carpenter and Morton described an improved medium for the isolation of the gonococcus in 24 hours.[1] The efficiency of this medium, GC Agar supplemented with hemoglobin and yeast concentrate, was demonstrated in a study of twelve media then in use for the isolation of this organism.[2] The medium was improved by replacing the yeast concentrate with **BBL™ IsoVitaleX™** Enrichment, a chemically defined supplement developed specially to aid the growth of gonococci, although it has broad application for other microorganisms; e.g., *Haemophilus*.[3,4] Through careful selection and pretesting of raw materials, Chocolate II prepared plated medium promotes improved growth of gonococci and *Haemophilus* species. With most strains of *N. gonorrhoeae*, visible growth on primary isolation is seen after incubation of 18-24 hours.

The isolation of fastidious organisms from specimens containing mixed flora is facilitated by selective agents. Bacitracin has been recommended for isolation of *Haemophilus* from the respiratory tract.[5,6]

Chocolate II Agar is often used as the medium for subculture from blood culture bottles to detect the presence of bacteria in cases of septicemia. Some cases of septicemia are caused by organisms referred to as "nutritionally variant streptococci." These organisms require certain forms of vitamin B_6, such as pyridoxal or pyridoxamine, and will not be isolated by the use of unsupplemented media.[7] Chocolate II Agar supplemented with pyridoxal may be used for this purpose.

Principles of the Procedure

Chocolate II Agar contains an improved GC Agar base (GC II Agar Base), bovine hemoglobin and **IsoVitaleX** Enrichment.



GC II Agar Base contains nitrogenous nutrients in the form of casein and meat peptones, phosphate buffer to maintain pH and corn starch, which neutralizes toxic fatty acids that may be present in the agar. Hemoglobin provides X factor (hemin) for *Haemophilus* species. **IsoVitaleX** Enrichment is a defined supplement which provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, co-enzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

Chocolate II Agar with Bacitracin is a selective medium for the isolation of *Haemophilus* species from specimens containing mixed flora. Bacitracin is a polypeptide antibiotic that inhibits gram-positive bacteria and *Neisseria*.[8]

Chocolate II Agar with Pyridoxal is an enriched medium that supports the growth of nutritionally variant streptococci, as well as *Haemophilus* and other fastidious microorganisms. Pyridoxal is a vitamin B_6 compound that is required for the growth of certain strains of streptococcus.[7]

Procedure

Chocolate II Agar

Streak the specimen as soon as possible after it is received in the laboratory. Place the culture in an aerobic environment enriched with carbon dioxide. Incubate at 35 ± 2°C and examine after overnight incubation and again after approximately 48 hours. Subcultures for identification of *N. gonorrhoeae* should be made within 18-24 hours.

Chocolate II Agar slants primarily are used for the cultivation and maintenance of pure cultures. The slants should be inoculated with a pure culture.

Chocolate II Agar with Bacitracin and Chocolate II Agar with Pyridoxal

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate plates at 35 ± 2°C for 18-24 hours in an aerobic atmosphere enriched with 5-10% carbon dioxide.

Expected Results

Chocolate II Agar

Typical colonial morphology on Chocolate II Agar is as follows:

Haemophilus influenzae.....Small (1 mm), moist, pearly with a characteristic "mousy" odor
Neisseria gonorrhoeae .....Small, grayish-white to colorless, mucoid
Neisseria meningitidis..... Medium to large, blue-gray, mucoid

Chocolate II Agar with Bacitracin and Chocolate II Agar with Pyridoxal

After a minimum of 18 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Haemophilus may appear as small (1 mm), moist, pearly colonies with a characteristic "mousy" odor.

References

1. Carpenter and Morton. 1947. Proc. N.Y. State Assoc. Public Health Labs. *27*:58.
2. Carpenter, Bucca, Buck, Casman, Christensen, Crowe, Drew, Hill, Lankford, Morton, Peizer, Shaw and Thayer. 1949. Am. J. Syphil. Gonorrh. Venereal Diseases *33*:164.
3. Martin, Billings, Hackney and Thayer. 1967. Public Health Rep. *82*:361
4. Vastine, Dawson, Hoshiwara, Yonega, Daghfous and Messadi. 1974. Appl. Microbiol. *28*:688.
5. Chapin and Doern. 1983. J. Clin. Microbiol. *17*:1163.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
7. Reimer and Reller. 1981. J. Clin. Microbiol. *14*:329.
8. Garrod and O'Grady. 1971. Antibiotics and chemotherapy, 3rd ed. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Chocolate II Agar (GC II Agar with Hemoglobin and IsoVitaleX™)

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	221169	Prepared Plates – Pkg. of 20*
	221267	Prepared Plates – Ctn. of 100*
	295872	Prepared Slants (K Tubes) – Pkg. of 10*

Japan

Cat. No.	251169	Prepared Plates – Pkg. of 20*
	251267	Prepared Plates – Ctn. of 100*
	251578	Prepared Plates – Ctn. of 200*

BBL™ Chocolate II Agar with Bacitracin

United States and Canada

Cat. No.	296271	Prepared Plates – Pkg. of 20*

Japan

Cat. No.	256271	Prepared Plates – Pkg. of 20*

BBL™ Chocolate II Agar with Bacitracin//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Japan

Cat. No.	251789	Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ Chocolate II Agar with Pyridoxal

Cat. No. 297259 Prepared Plates – Ctn. of 100*

BBL™ Chocolate II Agar//Martin-Lewis Agar

Cat. No. 297060 Prepared Bi-Plate Dishes – Pkg. of 20*
297245 Prepared Bi-Plate Dishes – Ctn. of 100*

BBL™ Chocolate II Agar//Modified Martin-Lewis Agar

Cat. No. 298513 Prepared Bi-Plate Dishes – Pkg. of 20*
298206 Prepared Bi-Plate Dishes – Ctn. of 100*

BBL™ Chocolate II Agar//Modified Thayer-Martin (MTM II) Agar

BS12 CMPH2 MCM9

Cat. No. 221623 Prepared Bi-Plate Dishes – Pkg. of 20*

BBL™ Chocolate II Agar//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

United States and Canada

Cat. No. 221302 Prepared **I Plate**™ Dishes – Pkg. of 20*
221303 Prepared **I Plate**™ Dishes – Ctn. of 100*

Europe

Cat. No. 251302 Prepared **I Plate**™ Dishes – Pkg. of 20*
251303 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ Chocolate II Agar//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//MacConkey II Agar

Cat. No. 299580 Prepared **Y Plate**™ Dishes – Ctn. of 100*

**Store at 2-8°C.*

Choline Assay Medium

Intended Use

Choline Assay Medium is used for determining choline concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimum growth of the test organism except the single essential vitamin to be determined.

Choline Assay Medium is a slight modification of the medium described by Horowitz and Beadle.[1] *Neurospora crassa* ATCC™ 9277 is the test organism used in this microbiological assay.

Principles of the Procedure

Choline Assay Medium is a choline-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *N. crassa* ATCC 9277. The addition of choline standard in specified increasing concentrations gives a growth response by this organism that can be measured gravimetrically.

Formula

Difco™ Choline Assay Medium

Approximate Formula* Per Liter		
Sucrose	40.0	g
Ammonium Nitrate	2.0	g
Potassium Sodium Tartrate	11.4	g
Monopotassium Phosphate	2.0	g
Magnesium Sulfate	1.0	g
Sodium Chloride	0.2	g
Calcium Chloride	0.2	g
Ferrous Sulfate	1.1	mg
Zinc Sulfate	17.6	mg
Biotin	10.0	µg
Sodium Borate	700.0	µg
Ammonium Molybdate	500.0	µg
Cuprous Chloride	300.0	µg
Manganese Sulfate	110.0	µg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 5.7 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense 10 mL amounts into flasks, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust flask volume to 20 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

User Quality Control

Identity Specifications

Difco™ Choline Assay Medium

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	2.85% (single strength) solution, soluble in purified water upon boiling. Solution is colorless, clear, may have a slight precipitate.
Prepared Appearance:	Colorless, clear, may have a slight precipitate.
Reaction of 2.85% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

Difco™ Choline Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Neurospora crassa* ATCC™ 9277 when prepared in single strength and supplemented with choline chloride. The medium should produce a standard curve when tested using a choline chloride reference standard at 0.0 to 25.0 µg per 10 mL. Incubate flasks with caps loosened at 25-30°C for 3 days. Measure the growth response gravimetrically – weight of mycelia versus micrograms of choline chloride standard.

Procedure

Remove 1 loop of spores from a 48-72 hour culture of *N. crassa* ATCC 9277 grown on Neurospora Culture Agar (per liter: Proteose Peptone No. 3, 5.0 g; Yeast Extract, 5.0 g; Maltose, 40.0 g; Agar, 15.0 g; pH 6.7 ± 0.2) and suspend it in 100 mL sterile saline. Add 1 drop of this spore suspension to each flask of medium. Incubate at 25-30°C for 3 days. At the end of the incubation period, steam the flask at 100°C for 5 minutes. Remove all the mycelium from the flask using a stiff wire needle or glass rod, press dry between paper towels, and roll into a small pellet. Dry the pellet at 100°C in a vacuum oven for 2 hours. (A glazed porcelain spot plate is convenient for handling the mycelium during drying and weighing.) Weigh to the nearest 0.5 mg. A standard curve is then constructed from the weights obtained, and the unknown determined by interpolation. In the assay for choline, 50 mL Erlenmeyer flasks containing a total volume of 10 mL each are used.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using choline at levels of 0.0, 2.5, 5, 10, 15, 20 and 25 µg per assay flask (10 mL). The most effective assay range using Choline Assay Medium is between 2.5 and 30 µg choline.

The concentration of choline required for the preparation of the standard curve may be prepared by dissolving 0.5 g choline chloride in 1,000 mL purified water. This is the stock solution (500 µg per mL). Dilute the stock solution by adding 1 mL to 99 mL purified water. Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL of this diluted solution per flask. Prepare the stock solution fresh daily.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

Reference

1. Horowitz and Beadle. 1943. J. Biol. Chem. *150*:325.

Availability

Difco™ Choline Assay Medium

Cat. No. 246010 Dehydrated – 100 g*

**Store at 2-8°C.*

Chopped Meat Carbohydrate Broth, PR II
Chopped Meat Glucose Broth, PR II

Intended Use

Chopped Meat Carbohydrate Broth, PR II and Chopped Meat Glucose Broth, PR II are pre-reduced media used in the enrichment, cultivation and maintenance of anaerobic microorganisms, particularly obligate anaerobes.

Summary and Explanation

These media utilize Hungate's method for culturing anaerobic microorganisms outside of an anaerobic chamber.[1] The tubes provide a reduced medium in a self-contained, anaerobic culture chamber sealed using a Hungate screw cap. The cap contains a butyl rubber septum stopper that permits inoculation and incubation outside an anaerobic chamber without exposing the medium to air.

They are recommended for subculture and enrichment of anaerobic isolates for chromatographic analysis and tests to determine proteolysis (meat digestion), spore formation, motility and toxin production, particularly by *Clostridium* species, and as a holding or stock culture maintenance medium.[2-4]

Principles of the Procedure

Pre-reduced medium provides an anaerobic nitrogen and hydrogen atmosphere. The tubes are packaged under oxygen-free conditions and sealed to prevent aerobiosis.

Meat pellets and enzymatic digest of casein provide amino acids and other nitrogenous substances to support bacterial growth. Yeast extract primarily provides the B-complex vitamins, and the phosphate is incorporated to maintain the pH of the medium. Carbohydrates provide energy sources. Hemin and vitamin K_1 are required by certain anaerobic species for growth and may improve the growth of other species.[5]

The reducing action of the meat particles and L-cysteine binds molecular oxygen. The reducing agents are required to maintain a low Eh. Resazurin is an oxidation-reduction indicator used to detect changes in the Eh of the medium. The medium remains colorless if the Eh remains low; increased oxidation causes the medium to become pink.

Procedure

Before inoculating, disinfect the septum of the cap. To inoculate, insert needle through the septum and inject the specimen into the medium. Withdraw the needle slowly to avoid introducing air into the tube.

Organisms for subculture should first be isolated in pure culture on an appropriate solid medium. Prepare a suspension of the pure culture in 0.5 to 1.0 mL of sterile, reduced broth and inoculate with one or two drops.

For enrichment purposes, inoculate the pre-reduced medium with one or two drops of the specimen after inoculating appropriate primary plating media. Prepare tissues and other solid specimens by mincing and grinding the specimen in 0.5 to 1.0 mL sterile, reduced broth. For swab specimens, scrape the swab in sterile, reduced broth.

Incubate the tubes at 35 ± 2°C for up to a week before discarding as negative. For suspected actinomycoses, osteomyelitis, endocarditis and other serious infections, incubate the tubes for up to two weeks before discarding as negative.

Expected Results

Examine the medium for blackening of meat particles, indicating digestion. Consult references for information needed for chromatographic analyses and tests for indole production, toxin production and spore formation.[2-4, 6, 7]

References

1. Hungate. 1969. Methods of microbiology. Academic Press, New York, N.Y.
2. Moore, Cato and Moore (ed.). 1987. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
3. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. Gibons and MacDonald. 1960. J. Bacteriol. *80*:164.
6. Summanen, Baron, Citron, Strong, Wexler and Finegold. 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing Co., Belmont, Calif.
7. Rodloff, Applebaum and Zabransky. 1991. Cumitech 5A, Practical anaerobic bacteriology. Coordinating ed., Rodloff. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Chopped Meat Carbohydrate Broth, PR II

CMPH2

Cat. No. 297307 Prepared Tubes (Hungate cap) – Pkg. of 10

BBL™ Chopped Meat Glucose Broth, PR II

Cat. No. 297305 Prepared Tubes (Hungate cap) – Pkg. of 10

CHROMagar™ Candida

Intended Use

BBL™ CHROMagar™ Candida* medium is for the isolation and differentiation of *Candida albicans, C. tropicalis* and *C. krusei*.[1] Due to the differences in morphology and colors of the yeast colonies, this medium facilitates the detection of mixed yeast cultures in specimens.[2,3] It may also be used as a selective isolation medium for other yeasts and for filamentous fungi instead of Sabouraud Dextrose Agar or similar media.

**U.S. Patent Nos. 5,716,799 and 5,962,251*

Summary and Explanation

The usefulness of a selective and differential medium for the primary isolation of *Candida* species has long been noted. In 1953, Nickerson developed a medium following a study of sulfite reduction by *Candida* species.[4] In 1958, Pagano et al. added triphenyl tetrazolium chloride to Sabouraud Dextrose medium to differentiate *C. albicans* from other yeasts.[5]

With the inclusion of chromogenic substrates in the medium, the colonies of *C. albicans, C. tropicalis* and *C. krusei* produce different colors, thus allowing the direct detection of these yeast species on the isolation plate.[1-3] Colonies of *C. albicans* appear light to medium green, *C. tropicalis* colonies appear dark blue to metallic-blue, and *C. krusei* colonies appear light rose with a whitish border. Other yeasts may develop either their natural color (cream) or appear light to dark mauve (e.g., *C. glabrata* and other species).

BBL™ CHROMagar™ Candida medium is a selective and differential medium deveolped by A. Rambach and is sold by BD under a licensing agreement with CHROMagar, Paris, France.

Principles of the Procedure

Specially selected peptones supply the nutrients in **BBL™ CHROMagar™** Candida medium. The chromogen mix consists of artificial substrates (chromogens), which release differently colored compounds upon degradation by specific enzymes. This permits the differentiation of certain species, or the detection of certain groups of organisms, with only a minimum of confirmatory tests. Chloramphenicol inhibits most bacterial contaminants.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates aerobically at 35 ± 2°C for 36-48 hours in an inverted position (agar-side up). Do not incubate in an atmosphere supplemented with carbon dioxide. Minimize exposure to light both before and during incubation. Occasional isolates, such as *Cryptococcus neoformans* and filamentous fungi, will require a longer incubation time and possibly a lower incubation temperature.

Expected Results

After incubation, plates from specimens containing yeasts will show growth. Depending on the yeast species, colonies will appear light to medium green (*C. albicans*), light rose to pink (*C. krusei*), dark blue to metallic blue (*C. tropicalis*), light to dark mauve or natural color (cream). Data from various studies indicate that further identification using biochemical and morphological tests for the above three yeast species is not necessary.[1-3] Colonies that appear light to dark mauve or appear in their natural cream color should be identified using standard methods.[6]

Limitations of the Procedure

1. Consult appropriate references for detailed information and recommended procedures for the identification of isolates.[1,3,6]
2. It has been reported that *C. dubliniensis* produces a distinctive dark green color on primary isolation with **BBL™ CHROMagar™** Candida medium.[7-9] However, this property may not be retained in subculture. Additional phenotypic and genotypic assays may be necessary. The clinical importance of *C. dubliniensis* requires further study.
3. Since molds and other filamentous fungi metabolize the chromogenic substrates, the colors exhibited by these organisms on **BBL™ CHROMagar™** Candida medium may differ from those exhibited on Sabouraud Dextrose Agar. Do not use the appearance of growth on this medium for traditional descriptive identification from Sabouraud Dextrose Agar.
4. Minimize exposure to light before and during incubation, as light may destroy the chromogens. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period.

References

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8. Kirkpatrick, Revankar, McAtee, Lopez-Ribot, Fothergill, McCarthy, Sanche, Cantu, Rinaldi and Patterson. 1998. J. Clin. Microbiol. *36*:3007.
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Availability

BBL™ CHROMagar™ Candida

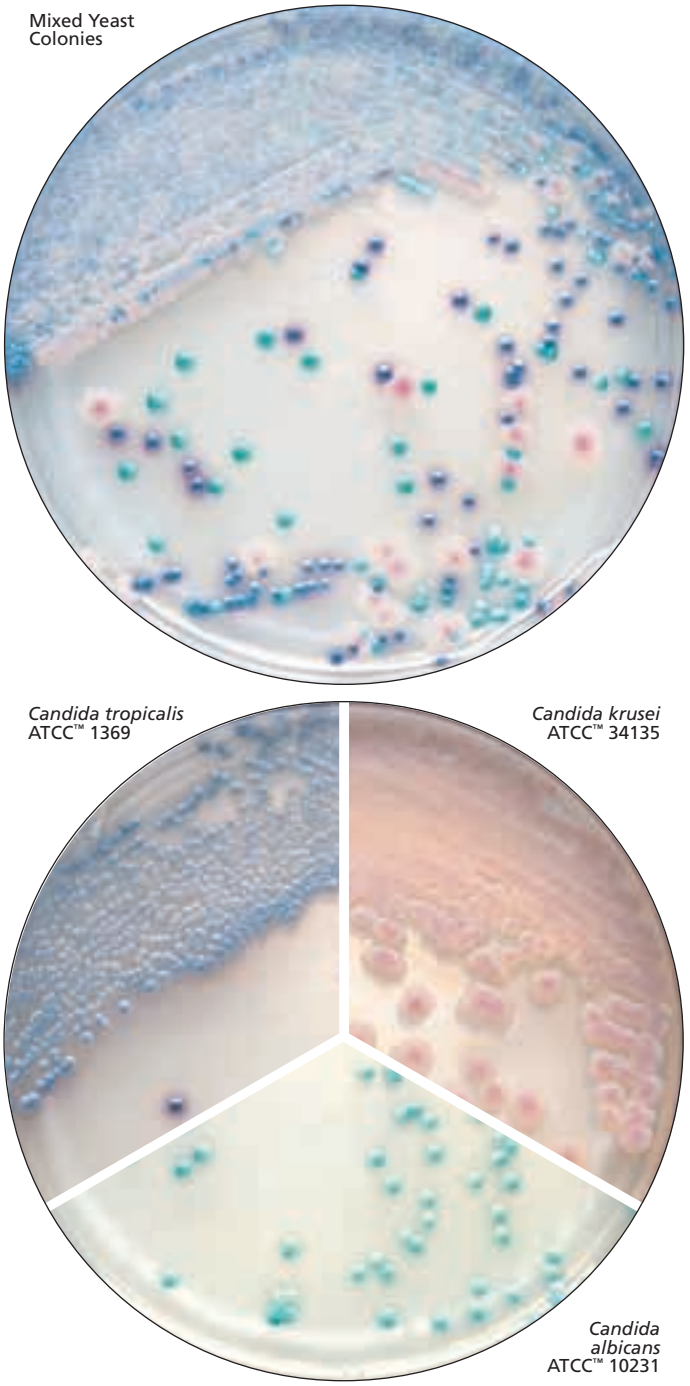
United States and Canada
Cat. No. 254093 Prepared Plates – Pkg. of 20*

Europe
Cat. No. 254106 Prepared Plates – Ctn. of 120*

Japan
Cat. No. 251594 Prepared Plates – Pkg. of 20*
251367 Prepared Plates – Ctn. of 100*

Mexico
Cat. No. 252630 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*



CHROMagar™ Listeria

Intended Use

BBL™ CHROMagar™ Listeria* is a selective medium for the isolation, differentiation and identification of *Listeria monocytogenes* and *L. ivanovii* from food and environmental samples.

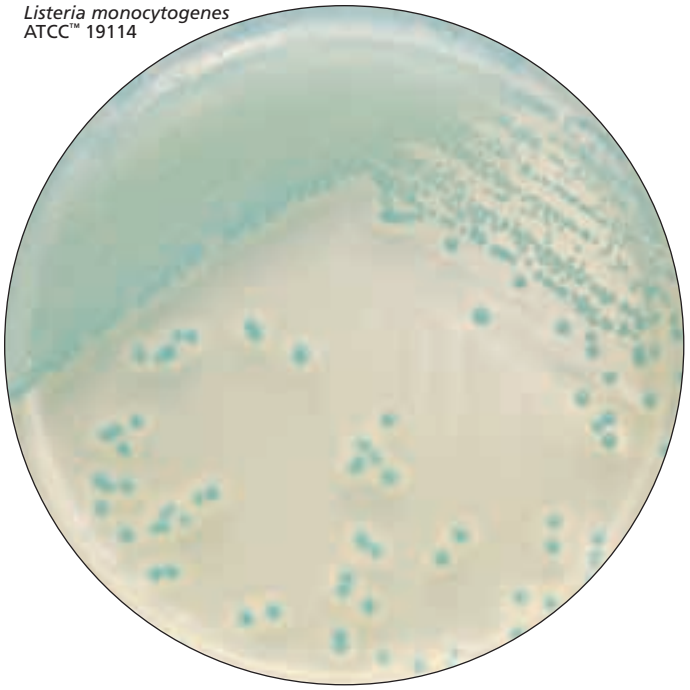
BBL CHROMagar Listeria has been validated by the AOAC™ Research Institute under the Performance Tested Methods℠ Program for the analysis of raw ground beef, smoked salmon, lettuce and Brie cheese when using FDA/BAM, USDA/FSIS, AOAC and ISO methods[1-4] with no confirmatory biochemical tests required for the identification of *Listeria monocytogenes*/*L. ivanovii*.

Confirmatory testing of isolates from food matrices other than those that have been validated, and from environmental samples, is recommended.

**U.S. Patent Pending*

C CHROMagar Listeria, cont.

Listeria monocytogenes
ATCC™ 19114



Summary and Explanation

Listeriosis is a foodborne illness caused by *L. monocytogenes*. It is of particular concern for immunocompromised patients: cancer, HIV, pregnant women, neonates and the elderly. Because of the severity of the disease, 20 deaths per 100 cases, listeriosis is a serious public health and agrifood industry concern. Illness caused by *L. monocytogenes* has been associated with deli meats, poultry, soft cheeses, ready-to-eat seafood, smoked fish, hot dogs, salad greens and inadequately or unpasteurized milk.[5,6] *L. ivanovii*, rarely found in foods, is pathogenic to animals and some cases of human listeriosis have been associated with this organism.[7]

BBL CHROMagar Listeria is intended for the isolation, differentiation and identification of *L. monocytogenes* and *L. ivanovii* based on the formation of blue-green colonies surrounded by an opaque, white halo. The addition of a chromogenic and a phospholipid substrate in the medium facilitates the detection and differentiation of *L. monocytogenes* and *L. ivanovii* from other *Listeria* species and organisms. An advantage **BBL CHROMagar** Listeria has over recommended traditional media, such as Modified Oxford and Oxford, is the ability to distinguish *L. monocytogenes* and *L. ivanovii* from other *Listeria* species. This facilitates the detection of *L. monocytogenes*/*L. ivanovii* in the presence of other *Listeria* species and other bacterial flora that may be present in a sample, thereby minimizing the risk of not detecting *L. monocytogenes* or *L. ivanovii*.

BBL CHROMagar Listeria has been validated by the AOAC Research Institute under the Performance Tested Methods Program.[8] It was evaluated for the detection of *Listeria monocytogenes* in raw ground beef, smoked salmon, lettuce and Brie cheese. The recovery of *L. monocytogenes* on **CHROMagar** Listeria was compared to the FDA/BAM, USDA/FSIS, AOAC and ISO reference plated media using the recommended pre-enrichments and selective enrichments. Of the 265 food samples tested, 140 were tested using BAM, USDA, or AOAC methods and 125 were tested using ISO methods. **BBL CHROMagar** Listeria produced a sensitivity of 99.3% and a specificity of 100% as compared to the reference methods for all food matrices. No false negatives were found in testing the food matrices. No statistical difference was found in recovery using the **CHROMagar** Listeria method compared to the reference plated media based on Chi square analysis. Furthermore, in the testing of raw ground beef and smoked salmon using ISO method 11290, time to recovery was shorter for **BBL CHROMagar** Listeria compared to ALOA.[9] Specifically, **BBL CHROMagar** Listeria recovered 27 positive samples from the primary (Half Fraser) and secondary (Fraser) broths after 24 hours of incubation compared to 3 positive samples detected on ALOA after 24 hours.[9] Finally, known isolates were evaluated and **CHROMagar** Listeria had a sensitivity and specificity of 100%. The results of these studies demonstrate that **BBL CHROMagar** Listeria is an effective medium for the recovery and detection of *Listeria monocytogenes* in raw ground beef, smoked salmon, lettuce and Brie cheese using FDA/BAM, USDA/FSIS, AOAC and ISO methods.

In a separate study, **BBL CHROMagar** Listeria was compared to AOAC[3] and Health Canada[10] reference methods using 50 natural and 150 spiked food samples comprising 50 different food types, including vegetables, milk, milk products, meat, seafood, poultry, ready-to-eat meats and mushrooms.[11] Overall, the sensitivity and specificity for **BBL CHROMagar** Listeria medium were 99% and 100%, respectively.[11] An additional study was performed comparing **BBL CHROMagar** Listeria to the USDA/FSIS *Listeria* isolation method using 63 poultry and environmental samples. **BBL CHROMagar** Listeria produced 100% (11/11) sensitivity and 100% (52/52) specificity. Modified Oxford medium was 100% sensitive; however, it lacked the ability to differentiate *L. monocytogenes* from other *Listeria* species.[11]

Principles of the Procedure

BBL CHROMagar Listeria was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar** Listeria prepared plated medium.

Specially selected **Difco**™ peptones supply nutrients. The addition of selective agents inhibits the growth of gram-negative organisms, yeast and fungi. The chromogen is a chromogenic substrate that produces a blue-green colored compound when hydrolyzed by an enzyme specific to *Listeria* species. A specific enzyme found in *L. monocytogenes* and *L. ivanovii* acts upon the phospholipid substrate in **BBL CHROMagar** Listeria producing an opaque, white halo around the blue-green colonies. The growth of a blue-green colony with well-defined edges surrounded by an opaque, white halo is presumptive for *L. monocytogenes* or *L. ivanovii* on **BBL CHROMagar** Listeria.

Sample Collection and Handling

Follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[1-4]

Procedure

Consult appropriate references and follow applicable standard methods. Inoculate the incubated enrichment broth sample onto a **BBL CHROMagar** Listeria plate and streak for isolation. Incubate plates aerobically at 35 ± 2°C for 24 hours. Do not incubate in CO_2. If negative, reincubate for an additional 24 hours to report final results.

Expected Results

After proper incubation, read plates against a white background. *L. monocytogenes/L. ivanovii* produce blue-green colonies with opaque, white haloes on **BBL CHROMagar** Listeria. *Listeria* species produce blue-green colonies without haloes. Gram-negative organisms are inhibited. Gram-positive organisms, other than *Listeria* species, will either be inhibited or produce white colonies.

Limitations of the Procedure

1. **BBL CHROMagar** Listeria cannot differentiate *L. monocytogenes* from *L. ivanovii* based on colony color or halo formation. Supplemental tests, such as hemolysis, xylose, rhamnose and CAMP or commercially available AOAC-RI approved *Listeria* identification biochemical test kits are necessary for differentiation of *L. monocytogenes* and *L. ivanovii.*
2. Incubation in CO_2 may adversely affect the recovery of *Listeria* species.

References

1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
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3. Horwitz (ed.). 2002. *Listeria monocytogenes* in milk and dairy products. In Official Methods of Analysis, AOAC International, 17th ed, AOAC International, Gaithersburg, Md.
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10. Health Canada. 2001/2002. The compendium of analytical methods, Health Products and Food Branch, Method MFHPB-30 and Supplement to the Method MFHPB-30.
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Availability

BBL™ CHROMagar™ Listeria

BAM

United States and Canada

Cat. No. 215085 Prepared Plates - Pkg. of 20*

Mexico

Cat. No. 252743 Prepared Plates - Pkg. of 10*

**Store at 2-8°C.*

CHROMagar™ MRSA

Intended Use

BBL™ CHROMagar™ MRSA* is a selective and differential medium for the qualitative direct detection of nasal colonization by methicillin-resistant *Staphylococcus aureus* (MRSA) to aid in the prevention and control of MRSA infections in healthcare settings. The test is performed on anterior nares swab specimens from patients and healthcare workers to screen for MRSA colonization. **BBL CHROMagar** MRSA is not intended to diagnose MRSA infection nor to guide or monitor treatment for infections.

**Patent Pending*

Summary and Explanation

MRSA is a major cause of nosocomial and life threatening infections. Infections with MRSA have been associated with a significantly higher morbidity, mortality and costs than methicillin-susceptible *S. aureus* (MSSA).[1] Selection of these organisms has been greatest in the healthcare setting; however, MRSA has also become more prevalent in the community.[2]

To control the transmission of MRSA, the Society for Healthcare Epidemiology of America (SHEA) has recommended guidelines, which include an active surveillance program to identify potential reservoirs and a rigorous infection control program to control the spread of MRSA.[1]

BBL CHROMagar MRSA is a selective and differential medium, which incorporates cefoxitin, for the detection of MRSA from anterior nares specimens.

BBL CHROMagar MRSA was developed by A. Rambach and BD. This product utilizes **CHROMagar** Staph aureus, which was developed by A. Rambach and is sold by BD under a licensing agreement with CHROMagar, Paris, France.

Principles of the Procedure

BBL CHROMagar MRSA medium permits the direct detection and identification of MRSA through the incorporation of specific chromogenic substrates and cefoxitin. MRSA strains will grow in the presence of cefoxitin[3] and produce mauve-colored colonies resulting from hydrolysis of the chromogenic substrate. Additional selective agents are incorporated for the suppression of gram-negative organisms, yeast and some gram-positive cocci. Bacteria other than MRSA may utilize other chromogenic substrates in the medium resulting in blue to blue/green colored colonies or if no chromogenic substrates are utilized, the colonies appear as white or colorless.

24 Hours Incubation	Interpretation/Recommended Action
Mauve colonies morphologically resembling staphylococci*	MRSA detected, report MRSA nasal colonization
No mauve colonies	No results available, reincubate 24 additional hours

48 Hours Incubation	Recommended Action	Interpretation
Mauve colonies	Perform coagulase testing	If coagulase positive – MRSA detected, report MRSA nasal colonization If coagulase negative – report no MRSA detected
No mauve colonies	N/A	Report no MRSA detected

** Staphylococci typically produce moderately sized smooth mauve colonies on **BBL CHROMagar** MRSA medium. Mauve colonies which are very small to pinpoint are most often gram-positive rods, usually corynebacteria. If morphology is unclear, confirmatory tests such as coagulase may be used to confirm identification at 48 hours.*

Staphylococcus aureus
ATCC™ 43300

Procedure

As soon as possible after receipt in the laboratory, inoculate the specimen onto a **BBL CHROMagar** MRSA plate and streak for isolation. Incubate plates aerobically at 35–37°C for 24 ± 4 hours in an inverted position. If no mauve colonies are recovered, reincubate for an additional 24 ± 4 hours. Do not incubate in an atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation as light may result in reduced recovery and/or coloration of isolates. Exposure to light is permissible after colony color develops.

Expected Results

Read plates against a white background. Colonies of MRSA will appear mauve on the **BBL CHROMagar** MRSA medium. Other organisms (non-MRSA) will be inhibited or produce colorless, white, blue or blue/green colonies. Refer to the table below for interpretation of results.

Limitations of the Procedure

1. Minimize exposure (<4 hours) of **BBL CHROMagar** MRSA to light both before and during incubation, as prolonged exposure may result in reduced recovery and/or coloration of isolates. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period.
2. At 48 hours occasional strains of coagulase-negative staphylococci (such as, *S. epidermidis, S. cohnii, S. intermedius, S. haemolyticus, S. capitis, S. hominis* and *S. schleiferi*), *Acinetobacter* sp., *Corynebacterium* and yeast may produce mauve-colored colonies requiring a confirmatory coagulase test for confirmation of MRSA. This may also occur at a much lower rate at 24 hours. In clinical studies, approximately 5% (6/120) of the mauve-colored colonies detected at 24 hours were coagulase-negative staphylococci and/or corynebacteria on the **BBL CHROMagar** MRSA medium. If desired, a coagulase test may be performed at 24 hours on mauve-colored colonies to increase specificity.
3. Surveillance testing determines the colonization status at a given time and could vary depending on patient treatment (e.g. decolonization regime), patient status (e.g. not actively shedding MRSA) or exposure to high risk environments (e.g. contact with MRSA carrier, prolonged hospitalization). Monitoring colonization status should be done according to hospital policies.
4. Results from **CHROMagar** MRSA should be used as an adjunct to nosocomial infection control efforts to identify patients needing enhanced precautions. The test is not intended to identify patients with staphylococcal infection. Results should not be used to guide or monitor treatment for MRSA infections. This device can be used to identify patients for isolation or removal from isolation to control nosocomial transmission of MRSA.
5. A **CHROMagar** MRSA negative result following a previous positive test result may indicate treatment eradication success or may occur due to intermittent shedding.
6. *mec*A-negative *S. aureus* may grow if the oxacillin or cefoxitin MICs are at or near the resistant breakpoint.
7. Incubation in 5% CO_2 is not recommended and may result in false negative cultures.
8. Use of phenylephrine hydrochloride, a component of some nasal sprays, at a concentration of ≥10% shows an inhibitory effect on organism growth that is unrelated to medium performance.

9. Rare strains of MRSA have demonstrated sensitivity to the **CHROMagar** MRSA base. This sensitivity is unrelated to methicillin resistance, but is due to a component in the base. As a result, these strains may appear as falsely susceptible to methicillin.

References

1. Muto, et al. 2003. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus*. Infect. Cont. Hospital Epidemiol. May 362.
2. Bannerman, T. L. 2003. *Staphylococcus, Micrococcus*, and other catalase-positive cocci that grow aerobically. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Yolken (eds.), 8th ed., Manual of clinical microbiology. American Society for Microbiology, Washington D.C.
3. Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial susceptibility testing; 18th Informational Supplement, M100-S18. CLSI, Wayne, Pa.

Availability

BBL™ CHROMagar™ MRSA

United States and Canada

Cat. No.	215084	Prepared Plates – Pkg. of 20 plates*
	215181	Prepared Plates – Ctn. of 100 plates*

Mexico

Cat. No.	252742	Prepared Plates – Pkg. of 10 plates*

**Store at 2-8°C.*

C

CHROMagar™ MRSA II

Intended Use

BBL™ CHROMagar™ MRSA II is a selective and differential medium for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimens. The test can be performed on nares and alternative body sites. For more product information, contact your local BD office (Europe only).

Availability

BBL™ CHROMagar™ MRSA II

Europe only

Cat. No.	257434	Prepared Plates – Pkg. of 20*
	257435	Prepared Plates – Ctn. of 120*

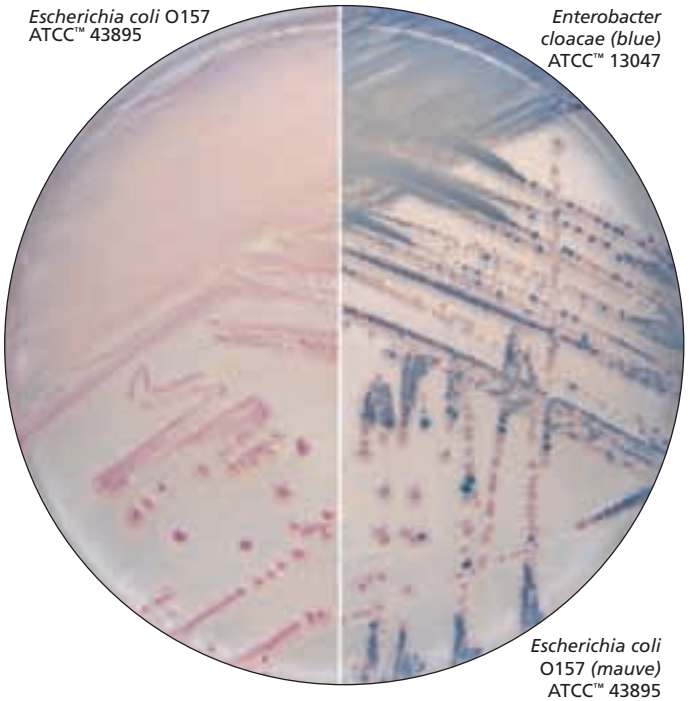
**Store at 2-8°C.*

CHROMagar™ O157

Intended Use

BBL™ CHROMagar™ O157* is a selective medium for the isolation, differentiation and presumptive identification of *Escherichia coli* O157:H7 from human clinical stool specimens.

BBL™ CHROMagar™ O157 is a selective medium for the isolation, differentiation and presumptive identification of *Escherichia coli* O157:H7 from food, veterinary and environmental sources and has been validated by the AOAC™ Research Institute under the Performance Tested Methods℠ Program for the analysis of raw ground beef and unpasteurized apple cider.



**U.S. Patent No. 6,165,743.*

Summary and Explanation

E. coli O157:H7 is the most frequently isolated pathogen from bloody stools.[1-3] However, absence of bloody diarrhea does not rule out the presence of *E. coli* O157:H7.[4] This serotype causes a broad range of illness from mild non-bloody diarrhea to severe bloody diarrhea (hemolytic colitis), hemolytic uremic syndrome and death.[1-3] The isolation of *E. coli* O157:H7 exceeds that of some other common enteric pathogens, especially *Shigella* in many areas and age groups. Transmission most often occurs through ingestion of raw or undercooked beef; other foods have also been implicated.[1,2] In addition, transmission may occur person to person, as well as from recreational water sources.[1,2]

CHROMagar O157 is intended for the isolation, differentiation and presumptive identification of *E. coli* O157:H7. Due to the chromogenic substrates in the medium, colonies of *E. coli* O157:H7 produce a mauve color, thus allowing presumptive identification from the primary isolation plate and differentiation from other organisms. In samples with low numbers of *E. coli* O157:H7, enrichment methods may be helpful prior to inoculating medium.

BBL CHROMagar O157 has been validated by the AOAC-Research Institute under the Performance Tested Methods Program.[5] **BBL CHROMagar** O157 was evaluated for the detection

of *E. coli* O157:H7 in raw ground beef and unpasteurized apple cider using seeded samples. The recovery of *E. coli* O157:H7 on **BBL CHROMagar** O157 was compared to the FDA/BAM, USDA/FSIS and ISO reference plated media. The reference recommended enrichment and screening procedures were followed for the reference media and **BBL CHROMagar** O157. Immunomagnetic separation (IMS) was performed according to the USDA and ISO methods. Of the 180 food samples tested, 45 were tested using FDA BAM and USDA FSIS methods, and 90 were tested using ISO methods. **BBL CHROMagar** O157 produced a sensitivity of 100% and a specificity of 100% as compared to the reference methods for both food matrices. No false negatives were found in testing the food matrices. No statistical difference was found in recovery using the **BBL CHROMagar** O157 method compared to the reference plated media based on Chi-square analysis. Known isolates, including 54 strains of *E. coli* O157:H7 (three of which were non-motile strains) and 32 non-*E. coli* O157:H7 strains, were evaluated on **BBL CHROMagar** O157 with a sensitivity and specificity of 100%. The results of these studies demonstrate that **BBL CHROMagar** O157 is an effective medium for the recovery and detection of *E. coli* O157:H7 in raw ground beef and unpasteurized apple cider using FDA BAM, USDA FSIS and ISO methods.

Principles of the Procedure

CHROMagar O157 was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of **BBL CHROMagar** O157 prepared plated medium.

Specially selected **Difco**™ peptones supply the nutrients. The addition of potassium tellurite, cefixime and cefsulodin reduces the number of bacteria other than *E. coli* O157:H7 that grow on this medium. The chromogen mix consists of artificial substrates (chromogens), which release an insoluble colored compound when hydrolyzed by a specific enzyme. *E. coli* O157:H7 utilizes one of the chromogenic substrates producing mauve colonies. The growth of mauve colonies is considered presumptive for *E. coli* O157:H7 on **BBL CHROMagar** O157. Non-*E. coli* O157:H7 bacteria may utilize other chromogenic substrates resulting in blue to blue-green colored colonies or, if none of the chromogenic substrates are utilized, colonies may appear as their natural color. This facilitates the detection and differentiation of *E. coli* O157:H7 from other organisms.

Sample Collection and Handling

For clinical specimens, refer to lab procedures for details on specimen collection and handling procedures.

For agrifood or other industrial samples, follow appropriate standard methods for details on sample preparation and processing according to sample type and geographic location.

Procedure

For clinical specimens, as soon as possible after receipt in the laboratory, inoculate onto a **BBL CHROMagar** O157 plate and streak for isolation. If the specimen is cultured from a swab, roll the swab over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2°C for 18-24 hours in an inverted position (agar-side up). Plates are not to be incubated beyond the 24-hour time period prior to reading. Interpretation of plate results must be completed within 18-24 hours after inoculation of the **BBL CHROMagar** O157 plate.

For food samples, consult appropriate references[6-8] and follow applicable standard methods. Inoculate incubated enrichment broth or screened food sample particle onto **BBL CHROMagar** O157 and streak for isolation. Incubate plates aerobically at 35 ± 2°C for 18-24 hours in an inverted position (agar-side up).

Expected Results

After proper incubation, read plates against a white background. Interpretation of plate results must be completed within 18-24 hours after inoculation of the **BBL CHROMagar** O157 plate. *E. coli* O157:H7 will produce mauve-colored colonies on **BBL CHROMagar** O157 medium. All mauve colonies should be confirmed biochemically and/or serologically prior to reporting as *E. coli* O157:H7.[3,6-8] Gram-positive organisms should be completely inhibited. Gram-negative organisms, other than *E. coli* O157:H7, will either be inhibited or produce colorless, blue, green, blue-green (aqua) or natural color colonies.

Limitations of the Procedure

1. **BBL CHROMagar** O157 does not detect enterohemorrhagic or enteropathogenic serotypes of *E. coli* other than O157:H7, since they may differ biochemically. β-glucuronidase-positive strains of *E. coli* O157:H7 will not be detected on **BBL CHROMagar** O157; however, such strains are rare.
2. **BBL CHROMagar** O157 does not differentiate between toxin-producing and non-toxin-producing strains of *E. coli* O157:H7.
3. Organisms other than *E. coli* O157:H7, such as *Proteus* spp. may grow on this medium; however, they generally produce a different color. If unisolated mauve colonies are observed, isolation can be achieved by subculturing to another **BBL CHROMagar** O157 plate. Rare strains of *E. coli* (biochemically similar to *Shigella*) have been found that produce false positive results on **BBL CHROMagar** O157.
4. Confirmatory tests are necessary for definitive identification.[3,6-8]
5. Incubation at lower than recommended temperatures may delay detection of positive reactions. If the incubation temperature is below 35 ± 2°C, the plates should be incubated a full 24 hours before reporting as negative.[9]
6. Plates are not to be incubated beyond the 24-hour time period prior to reading.
7. For clinical specimens, internal cross reactivity testing has demonstrated that *Salmonella* serotype Heidelberg exhibited mauve colonies when plated on **BBL CHROMagar** O157 medium. As recommended, all mauve colonies should be confirmed by biochemical or serological testing prior to reporting results.

References

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9. Data on file, BD Diagnostics.

Availability

BBL™ CHROMagar™ O157

CCAM

United States and Canada

Cat. No. 214984 Prepared Plates - Pkg. of 20*

Europe

Cat. No. 254105 Prepared Plates - Pkg. of 20*

Japan

Cat. No. 251361 Prepared Plates – Pkg. of 20*

251362 Prepared Plates – Ctn. of 100*

Mexico

Cat. No. 252717 Prepared Plates - Pkg. of 10*

**Store at 2-8°C.*

C

CHROMagar™ Orientation

Intended Use

BBL™ CHROMagar™ Orientation* medium is a nonselective medium for the isolation, differentiation and enumeration of urinary tract pathogens. **BBL CHROMagar** Orientation medium allows for the differentiation and identification of *Escherichia coli* and *Enterococcus* without confirmatory testing.

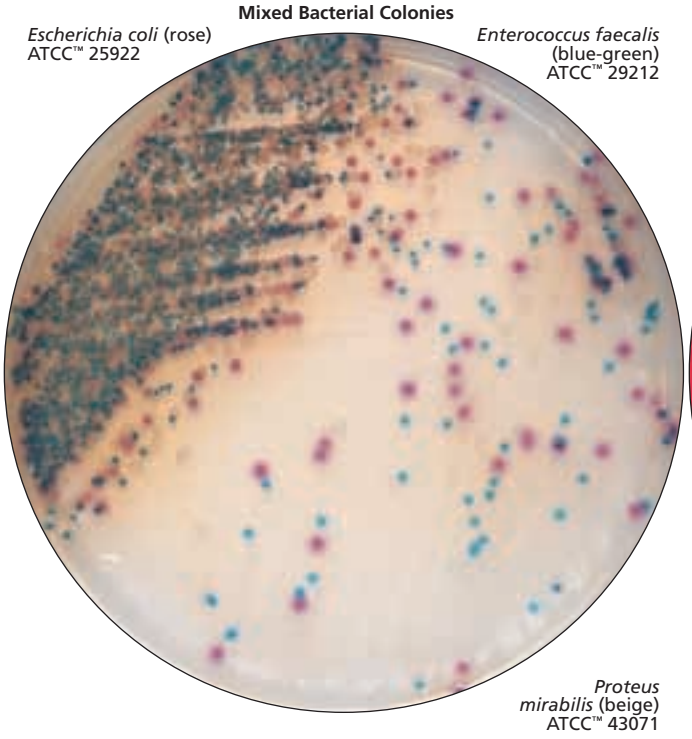
**U.S. Patent Nos. 5,716,799 and 5,962,251*

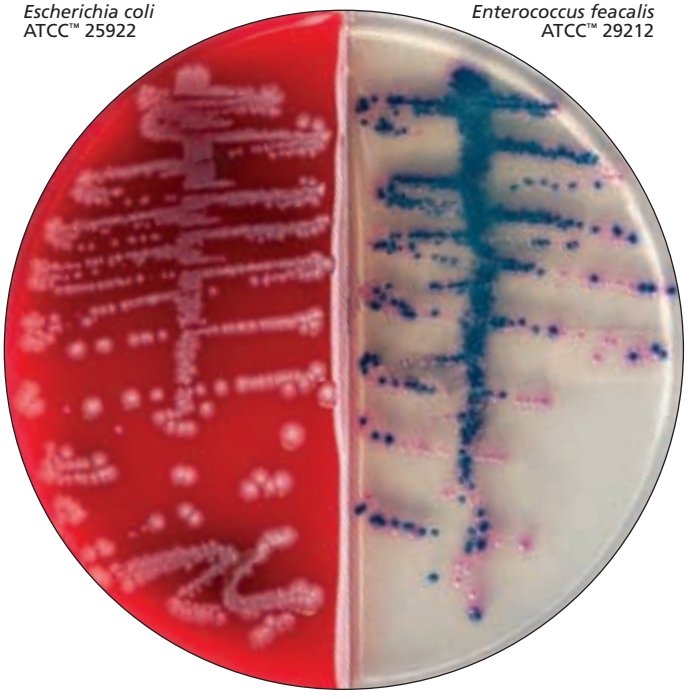
Summary and Explanation

Escherichia coli, enterococci, the *Klebsiella-Enterobacter-Serratia* (KES) and the *Proteus-Morganella-Providencia* (PMP) groups are frequently encountered organisms in urinary tract infections (UTI). Most UTIs are caused by *E. coli* alone, or in combination with enterococci. *Staphylococcus saprophyticus* and *Streptococcus agalactiae* may be isolated from females, although less frequently.

Due to the different antimicrobial susceptibility patterns of the microorganisms involved, identification to the species level is necessary for effective antimicrobial therapy. The most frequently isolated species or organism groups produce characteristic enzymes. Thus, it is possible to identify these organisms to the species level with a limited number of substrate fermentation or utilization tests.[1]

Some of the organisms encountered in UTIs produce enzymes either for the metabolism of lactose or glucosides or both. Other organisms produce none of these enzymes. For example, *E. coli* contains enzymes for lactose metabolism but is β-glucosidase negative. Some members of the family *Enterobacteriaceae* are β-glucosidase positive but do not contain enzymes necessary for lactose fermentation; others may contain both types of enzymes or none of them. β-glucosidases are also found in gram-positive cocci, such as *S. agalactiae* and enterococci.





Tryptophan deaminase (TDA) is an enzyme characteristically found in the *Proteus-Morganella-Providencia* group.

BBL™ CHROMagar™ Orientation medium was developed by A. Rambach and is sold by BD under a licensing agreement with CHROMagar, Paris, France.

Principles of the Procedure

Specially selected peptones supply the nutrients in **BBL™ CHROMagar™** Orientation medium. The chromogen mix consists of artificial substrates (chromogens) which release differently colored compounds upon degradation by specific microbial enzymes, thus assuring the differentiation of certain species or the detection of certain groups of organisms, with only a minimum of confirmatory tests. *Proteus* swarming is partially to completely inhibited.

Procedure

A dilution of the specimen on the plate (by using calibrated loops or other techniques commonly used for plating urine specimens) is required to obtain isolated colonies with typical colors and morphology. Incubate plates aerobically at 35 ± 2°C for not less than 20-24 hours in an inverted position (agar-side up). Do not incubate in an atmosphere supplemented with carbon dioxide. Avoid exposure to light during incubation as light may destroy the chromogens. Once the colony color develops, exposure to light is permissible.

Expected Results

After incubation, the plates should show isolated colonies in the areas where the inoculum was diluted appropriately.

Typical colony appearance on **BBL™ CHROMagar™** Orientation medium is as follows:

E. coli	Dark rose to pink, transparent colonies, with or without halos in the surrounding medium
KES group	Medium-blue to dark blue colonies
PMP group	Pale to beige colonies surrounded by brown halos*
Enterococcus	Blue-green small colonies
S. agalactiae	Blue-green to light blue, pinpoint to small colonies, with or without halos
S. saprophyticus (most strains)	Light pink to rose, small opaque colonies with or without halos
Other including yeasts	Natural (cream) pigmentation

** About 50% of* P. vulgaris *strains produce blue colonies on a brownish medium.*
Key: KES = Klebsiella-Enterobacter-Serratia *group;*
PMP = Proteus-Morganella-Providencia *group.*

Clinical studies have demonstrated that **BBL™ CHROMagar™** Orientation medium has advantages over other differential media used in the isolation, differentiation and enumeration of UTI pathogens, such as CLED Agar or a combination of Blood and MacConkey Agars.[2-4] **BBL™ CHROMagar™** Orientation medium allows the differentiation of *E. coli* and enterococci without confirmatory testing. Presumptive identification of *S. saprophyticus*, *S. agalactiae*, *Klebsiella-Enterobacter-Serratia* (KES) and the *Proteus-Morganella-Providencia* (PMP) groups is possible by means of colony morphology, pigmentation and medium discoloration.

Limitations of the Procedure

1. As this medium is nonselective, other UTI pathogens will grow. Colonies that show their natural color and do not react with the chromogenic substrates must be further differentiated with appropriate biochemical or serological tests to confirm identification.
2. *E. coli* colonies that are dark rose to pink but are pinpoint to small in size, require additional confirmatory tests such as spot indole (DMACA indole reagent).
3. Gram-negative organisms other than those belonging to the KES group may produce large blue colonies and thus require other biochemical tests for identification.
4. In very rare cases, *Listeria monocytogenes* or other *Listeria* species may be present in urine (e.g., after abortion due to these agents). *Listeria* will produce blue to blue-green colonies that are PYR-negative, mimicking *Streptococcus agalactiae*. Therefore, it may be useful to perform a Gram stain of organisms producing small, blue to blue-green colonies on this medium that are PYR negative. The presence of gram-positive bacilli may be indicative of *Listeria* species, but additional biochemical tests are necessary to confirm their identification.
5. Very rarely, isolates of *Aeromonas hydrophila* may produce rose colonies. They may be differentiated from *E. coli* with the oxidase test (*Aeromonas* is positive; *E. coli* is negative).
6. This medium will not support the growth of fastidious organisms, such as *Neisseria* spp., *Haemophilus* spp. or *Mycoplasma* spp.
7. Use of this medium for nonclinical or clinical specimens other than urine has not been documented.
8. Minimize exposure to light before and during incubation, as light may destroy the chromogens. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period.

References

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Availability

BBL™ CHROMagar™ Orientation

United States and Canada

Cat. No.	254102	Prepared Plates – Pkg. of 20*
	215081	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254107	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251781	Prepared Plates – Pkg. of 20*
	252086	Prepared Plates – Ctn. of 100*

Mexico

Cat. No.	252631	Prepared Plates – Pkg. of 10*

BBL™ CHROMagar™ Orientation//BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No.	222239	Prepared **I Plate**™ Dishes – Ctn. of 100*

**Store at 2-8°C.*

CHROMagar™ Salmonella

Intended Use

BBL™ CHROMagar™ Salmonella* is a selective and differential medium for the isolation and presumptive identification of *Salmonella* species from other coliform and non-coliform bacteria in clinical stool samples and a variety of food samples.

BBL™ CHROMagar™ Salmonella, prepared plated medium, and **Difco™ CHROMagar™** Salmonella, dehydrated culture medium, have been validated by the AOAC™ Research Institute under the Performance Tested Methods℠ program for the analysis of raw ground beef, raw chicken, raw fish, lettuce and shell eggs. ISO, USDA/FSIS and FDA/BAM methods were used for method comparison testing.[1-3] **CHROMagar** Salmonella was found to be equivalent to the plated media recommended in the ISO, FDA and USDA methods.

**U.S. Patent Nos. 5,098,832 and 5,194,374*

Summary and Explanation

Salmonella is ubiquitous in animal populations and is generally isolated from the intestinal tract of animals and humans. It is one of the most prevalent organisms associated with foodborne illnesses, which is often linked to animal origin.[4] Illnesses caused by *Salmonella* have been associated with poultry, beef, chocolate, dairy and vegetable products.[5]

CHROMagar Salmonella is intended for the isolation and differentiation of *Salmonella* species. The addition of chromogenic substrates in the medium facilitates detection of *Salmonella* species from other flora.

CHROMagar Salmonella was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar** Salmonella prepared plated medium using the **Difco CHROMagar** Salmonella dehydrated culture medium formulation.

CHROMagar Salmonella media (prepared plates and dehydrated) have been validated by the AOAC Research Institute under the Performance Tested Methods Program for testing a variety of food types, including raw ground beef, raw chicken, raw fish, lettuce and shell eggs.[6] The prepared plates and plates made from the dehydrated culture medium were compared to the USDA/FSIS and FDA/BAM reference methods. The prepared plates were also compared to the ISO reference media. **BBL CHROMagar** Salmonella prepared plates performed as well as the reference media in all of the food samples with 100% agreement for each of the three methods. The dehydrated

User Quality Control

Identity Specifications

Difco™ CHROMagar™ Salmonella

Dehydrated Appearance:	Free-flowing, homogeneous, very pale to light yellow to tan or very pale pink.
Solution:	3.74% solution, soluble in purified water upon boiling.
Prepared Appearance:	Light to medium yellow to tan or very pale pink with no significant precipitate.
Reaction of 3.74% Solution at 25°C:	pH 7.6 ± 0.2

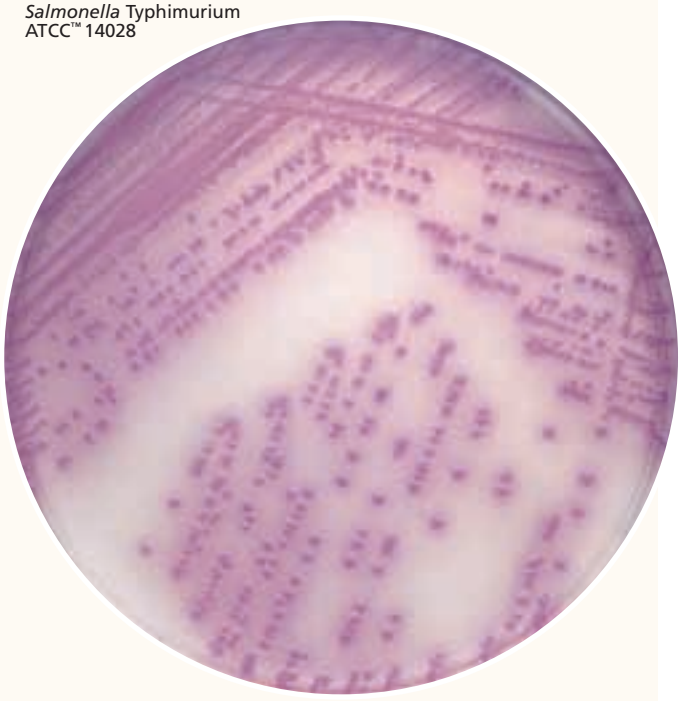
Cultural Response

Difco™ CHROMagar™ Salmonella

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours, up to 48 hours for *Salmonella* strains.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Citrobacter freundii	8090	10^4-10^5	Good	Blue to blue-green
Escherichia coli	25922	10^4-10^5	Partial to complete inhibition	Blue to green-blue
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10^3-10^4	Good	Mauve
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Mauve
Staphylococcus aureus	25923	10^4-10^5	Partial to complete inhibition	Cream

Salmonella Typhimurium ATCC™ 14028



medium also produced 100% agreement versus the FDA/BAM and USDA/FSIS reference media. The results of this study demonstrate that **BBL CHROMagar** Salmonella prepared plates and **Difco CHROMagar** Salmonella dehydrated culture medium are effective for the isolation and presumptive identification of *Salmonella* in raw chicken, raw ground beef, raw fish, lettuce and shell eggs.

In a separate study, Cox and Bailey compared **BBL CHROMagar** Salmonella to the USDA/FSIS reference methods for detection of *Salmonella* in rinses of whole chicken carcasses.[7] Based on 480 samples, **CHROMagar** Salmonella produced a sensitivity of 96.7% when compared to the two standard media combined (Brilliant Green Sulfa Agar and Modified Lysine Iron Agar). The researchers concluded that **CHROMagar** Salmonella is a feasible single plate method for the detection of *Salmonella* from whole chicken carcass rinses.[7]

Principles of the Procedure

Specially selected peptones supply the nutrients. Gram-positive organisms are generally inhibited as a result of the selective medium base. The addition of an antifungal agent prevents the growth of *Candida* species and other antimicrobial agents are used to inhibit the growth of gram-negative, non-glucose fermenting bacteria and *Proteus* species, which could potentially overgrow *Salmonella* colonies. A chromogenic mixture is included in the medium. Due to metabolic differences in the presence of selected chromogens, colonies of *Salmonella* species appear mauve (rose to purple) in color, whereas undesired bacteria are either inhibited, or produce blue-green or colorless colonies.

Formula

Difco™ CHROMagar™ Salmonella

Approximate Formula* Per Liter		
Chromopeptone	22.0	g
Chromogenic mix	0.34	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 37.4 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. DO NOT AUTOCLAVE. Cool to 45-50°C.
4. Aseptically add the following: Sodium Novobiocin (0.01 g/L), Cefsulodin (0.006 g/L), and Amphotericin B (0.004 g/L). If stock solutions are prepared, add 1 mL of each stock solution per liter of medium.
5. Mix well and dispense approximately 20 mL per Petri dish.
6. Immediately after plates have been poured and have solidified, protect from light. Store and incubate plates in the dark.
7. Test samples of the finished product for performance using stable, typical control cultures.

Directions for the Preparation of Antibiotic Stock Solutions

Novobiocin Stock Solution: Dissolve 0.2 g of novobiocin into 20 mL of purified water and filter sterilize into a sterile tube/bottle. Aseptically add 1 mL of stock solution per liter of medium.

Cefsulodin Stock Solution: Dissolve 0.3 g of cefsulodin into 50 mL of purified water and filter sterilize into a sterile tube/bottle. Aseptically add 1 mL of stock solution per liter of medium.

Amphotericin B Stock Solution: Dissolve 0.44 g of amphotericin B into 100 mL of purified water and filter sterilize into a sterile tube/bottle. Aseptically add 1 mL of stock solution per liter of medium.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.

Procedure

For clinical specimens, as soon as possible after receipt in the laboratory, inoculate the specimen onto a **CHROMagar** Salmonella plate and streak for isolation. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2°C in an inverted position (agar-side up) for 24 hours. If negative at 24 hours, reincubate for an additional 24 hours to report final results. Once the colony color develops, exposure to light is permissible. Typical colonies of *Salmonella* should be subjected to confirmatory biochemical or serological testing.

For food samples, follow sample preparation methodology as outlined in USDA/FSIS's *Microbiology Laboratory Guidebook: Isolation and Identification of* Salmonella *from Meat, Poultry, and Egg Products*, FDA/BAM's chapter on *Salmonella*, ISO guidelines or the procedure guidelines appropriate to sample type and geographic location.

Inoculate the incubated enrichment broth sample onto a **CHROMagar** Salmonella plate. Streak for isolation and incubate plates aerobically at 35 ± 2°C in an inverted position (agar side up) for 24 hours. If negative at 24 hours, reincubate for an additional 24 hours to report final results. Typical colonies of *Salmonella* growing on **CHROMagar** Salmonella should be subjected to confirmatory testing as outlined in ISO, USDA/FSIS and FDA/BAM procedures.[1-3]

Expected Results

After proper incubation, read plates against a white background. *Salmonella* Typhimurium and other *Salmonella* species will appear as light mauve to mauve-colored colonies, with the exception of *Salmonella enterica* subspecies *arizonae* and other *Salmonella* species positive for lactose and beta-glucosidase.

Those isolates will appear as blue-violet or purple colonies. *Citrobacter* and other coliforms will appear as light blue-green to blue-green colored colonies. Some organisms that do not hydrolyze any of the chromogenic compounds may appear as colorless colonies.

Limitations of the Procedure

1. Occasionally strains of *Aeromonas hydrophila, Hafnia alvei, Pseudomonas aeruginosa, P. putida, Acinetobacter* species, or *Candida* species may not be completely inhibited and colonies may exhibit light mauve to mauve pigmentation.
2. Confirmatory tests that use mauve or purple as an indicator color reaction may be difficult to interpret due to the actual colony color.
3. Rare strains of the following organisms: *S.* Typhi, *S.* Paratyphi A, *S.* Typhimurium, *S.* Choleraesuis, *S.* Minnesota, *S. enterica* subsp. *arizonae*, and *S.* Pullorum may fail to grow or have reduced growth on this medium. This is strain specific and the majority of the strains tested of each of these serovars were recovered.
4. **CHROMagar** Salmonella is not designed for the isolation of intestinal pathogens other than *Salmonella*. When testing some samples, a purple discoloration of the medium, without detectable colony growth, may be observed. This should be considered a negative result.
5. Minimize exposure of **CHROMagar** Salmonella to light before and during incubation, as light may destroy the chromogens. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period.
6. Incubation in CO_2 is not recommended.

References

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5. D'Aoust, Mauer and Bailey. 2001. *Salmonella* species. *In* Doyle, Beuchat and Montville (ed.), Food microbiology: fundamentals and frontiers, 2nd ed. American Society for Microbiology, Washington, D.C.
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Availability

Difco™ CHROMagar™ Salmonella

Cat. No. 214925 Dehydrated – 374 g (10 L)

BBL™ CHROMagar™ Salmonella

United States and Canada

Cat. No. 214983 Prepared Plates – Pkg. of 20*

Europe

Cat. No. 254104 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251356 Prepared Plates – Pkg. of 20*
251357 Prepared Plates – Ctn. of 100*
251364 Prepared **RODAC**™ Plates – Pkg. of 30*

Mexico

Cat. No. 252716 Prepared Plates – Pkg. of 10*

BBL™ CHROMagar™ Salmonella//BBL™ XLD Agar

Europe

Cat. No. 257372 Prepared **I Plate**™ Dishes – Pkg. of 20*

**Store at 2-8°C.*

CHROMagar™ Staph aureus

Intended Use

BBL™ CHROMagar™ Staph aureus* is a selective medium for the isolation, enumeration and identification of *Staphylococcus aureus* from clinical and food sources. Confirmatory testing of typical isolates from clinical sources is not required.

BBL™ CHROMagar™ Staph aureus (prepared plated medium) has been validated by the AOAC™ Research Institute under the Performance Tested Methods℠ Program for the analysis of shell eggs, smoked salmon and cooked roast beef when using AOAC and ISO methods.[1,2] Confirmatory testing of mauve-colored colonies obtained from the food matrices mentioned above is required.

**U.S. Patent No. 6,548,268*

Summary and Explanation

S. aureus is a well documented pathogen. It is responsible for infections ranging from superficial to systemic.[3,4] Due to the prevalence of this organism and its clinical implications, detection is of utmost importance.

Staphylococcal food poisoning caused by *S. aureus* is one of the most common types of foodborne illness worldwide. Its detection and enumeration help provide information about the potential health hazard of food, as well as being an indicator of poor hygiene.[5] It is also recommended that this organism be used as an indicator of water quality.[6]

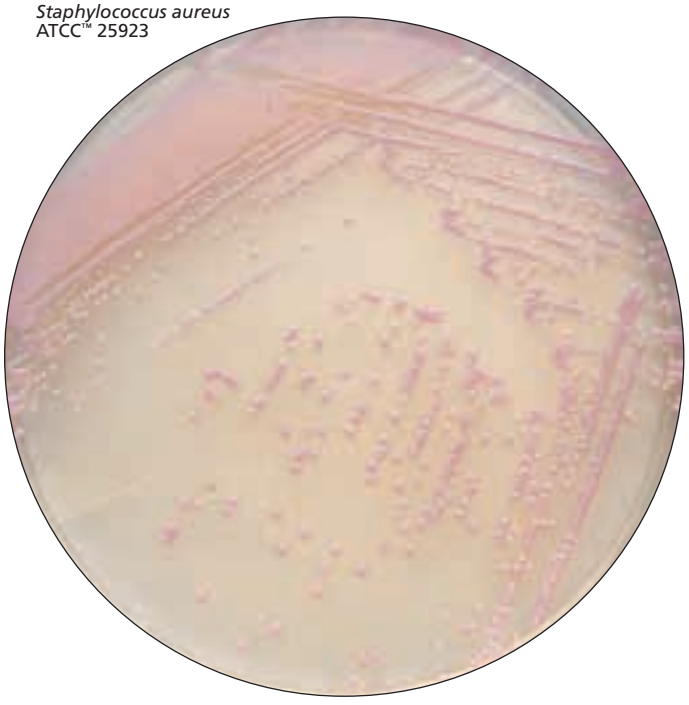
BBL CHROMagar Staph aureus is intended for the isolation, enumeration and identification of *S. aureus* based on the formation of mauve-colored colonies. The addition of chromogenic substrates to the medium facilitates the differentiation of *S. aureus* from other organisms. An advantage **BBL CHROMagar** Staph aureus has over some traditional media, such as Baird-Parker Agar, is the ability to identify *S. aureus* in 24 hours as opposed to 48 hours.

BBL CHROMagar Staph aureus has been validated by the AOAC Research Institute under the Performance Tested Methods Program.[7] The medium was evaluated for the recovery and enumeration of *S. aureus* in cooked roast beef, smoked salmon and shell eggs. The recovery and enumeration of *S. aureus* on

BBL CHROMagar Staph aureus was compared to the AOAC and ISO reference plated medium, Baird-Parker Agar, using the recommended diluents at low, medium and high inoculum levels of *S. aureus*. After 24 hours of incubation, enumeration was performed on **BBL CHROMagar** Staph aureus and after 48 hours on Baird-Parker Agar.

Based on statistical analysis, no significant difference was found between the reference methods and the **BBL CHROMagar** Staph aureus method for any food type or contamination level, with the exception of a low-level smoked salmon sample. The low contamination level of smoked salmon demonstrated a statistical difference in internal testing using the ISO method; i.e., the **BBL CHROMagar** Staph aureus method at 24 hours recovered more colonies ($\log_{10}$ 2.04) than the ISO reference at 48 hours ($\log_{10}$ 1.64). The repeatability precision estimates of the **BBL CHROMagar** Staph aureus method were satisfactory. The correlation coefficients ranged from 92.6% to 99.4%, demonstrating good correlation for all contamination levels in all food types. No false-positive colonies were recovered from the food matrices using **BBL CHROMagar** Staph aureus, and all mauve colonies were confirmed as *S. aureus* with no discrepancies. Known isolates, including 30 strains of *S. aureus* (several of which were enterotoxin-producing strains) and 37 non-*S. aureus* isolates were evaluated producing both a sensitivity and specificity of 100% on **BBL CHROMagar** Staph aureus. The results of these studies demonstrate that **BBL CHROMagar** Staph aureus can be used for the isolation, enumeration and presumptive identification of *S. aureus* in cooked roast beef, smoked salmon and shell eggs using AOAC and ISO methods.

Staphylococcus aureus
ATCC™ 25923



Principles of the Procedure

BBL CHROMagar Staph aureus was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar** Staph aureus prepared plated medium.

Specially selected **Difco**™ peptones supply nutrients. The addition of selective agents inhibits the growth of gram-negative organisms, yeast and some gram-positive cocci. The chromogen mix consists of artificial substrates (chromogens), which release an insoluble colored compound when hydrolyzed by specific enzymes. This facilitates the detection and differentiation of *S. aureus* from other organisms. *S. aureus* utilizes one of the chromogenic substrates, producing mauve-colored colonies. The growth of mauve-colored colonies at 24 hours is considered positive for *S. aureus* on **BBL CHROMagar** Staph aureus. Bacteria other than *S. aureus* may utilize other chromogenic substrates resulting in blue, blue-green, or if no chromogenic substrates are utilized, natural colored colonies.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.

Procedure

For clinical specimens, as soon as possible after receipt in the laboratory, inoculate onto a **BBL CHROMagar** Staph aureus plate and streak for isolation. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2°C for 20-24 hours in an inverted position (agar-side up).

For food samples, consult appropriate references and follow applicable standard methods. Inoculate the homogenized food samples onto **BBL CHROMagar** Staph aureus using the spread plate technique. Incubate plates aerobically at 35-37°C for 20-28 hours in an inverted position (agar-side up).

Expected Results

After proper incubation, read plates against a white background. *S. aureus* produces mauve to orange-mauve colored colonies on the **BBL CHROMagar** medium. Most gram-positive organisms, if not inhibited, will produce blue, blue-green or natural color (colorless, white or cream) colonies. Gram-negative organisms and yeasts are partially to completely inhibited.

Limitations of the Procedure

1. Occasional strains of staphylococci, other than *S. aureus*, such as *S. cohnii, S. intermedius* and *S. schleiferi*, as well as coryne-bacteria and yeasts, may produce mauve-colored colonies at 24 hours.[8] Differentiation of *S. aureus* from non-*S. aureus* can

be accomplished by coagulase, other biochemicals or Gram stain. Resistant gram-negative bacilli, which typically appear as small blue colonies, may also breakthrough.
2. Incubation beyond 24 hours (clinical) and 28 hours (food) is not recommended due to an increase in potential false positives. If incubation time is exceeded, mauve-colored colonies should be confirmed prior to reporting as *S. aureus*.
3. Incubation for less than the recommended 20 hours may result in a lower percentage of correct results being obtained.
4. Due to the natural golden pigment of some *S. aureus* strains, colony color may appear orange-mauve.

References

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8. Data on file, BD Diagnostics.

Availability

BBL™ CHROMagar™ Staph aureus

United States and Canada

Cat. No.	214982	Prepared Plates – Pkg of 20*

Europe

Cat. No.	257074	Prepared Plates – Pkg. of 20*
	257099	Prepared Plates – Ctn. of 120*

Mexico

Cat. No.	252715	Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

C

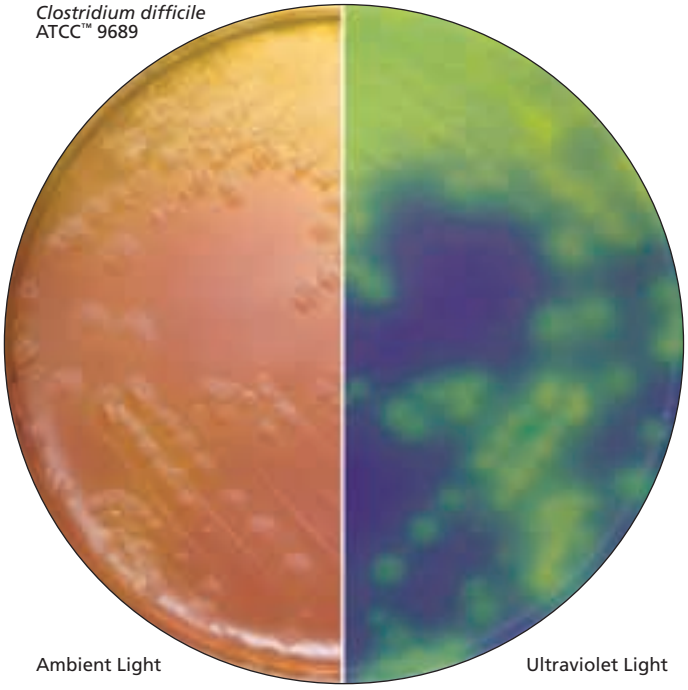
Clostridium Difficile Selective Agar

Intended Use

Clostridium Difficile Selective Agar (CDSA) is recommended as a selective and differential medium for the primary isolation of *Clostridium difficile* from fecal specimens.

Summary and Explanation

CDSA is a selective and differential medium developed by BD Diagnostic Systems. It permits superior recovery of *C. difficile*, with equivalent to better inhibition of normal flora when compared to CCFA (cycloserine-cetoxitin-fructose agar). As growth of *C. difficile* occurs, the pH of the medium is raised causing the neutral red indicator to turn yellow.



Principles of the Procedure

CDSA employs a peptone base with 0.6% mannitol. Ingredients have been optimized to improve recovery and colony size of *C. difficile*. Amino acids present in the agar base are utilized by *C. difficile* causing an increase in pH. The colony and surrounding medium change color from rose to yellow as the pH rises. Mannitol is utilized by fewer *Clostridium* species than fructose and improves the recovery of *C. difficile*. Cefoxitin and cycloserine are incorporated to inhibit normal fecal flora. These antibiotics have a broad range of antimicrobic activity against aerobic, anaerobic and facultatively anaerobic gram-positive and gram-negative bacteria while permitting recovery of *C. difficile*. *C. difficile* colonies produce yellow fluorescence when viewed with long-wave UV light.

Procedure

As soon as possible after receipt in the laboratory, inoculate the specimen onto a reduced CDSA plate and streak for isolation. As some strains of *C. difficile* may not grow well due to the selective properties of the medium, it is advisable to include a nonselective medium, such as CDC Anaerobe Blood Agar.

Media should be reduced prior to inoculation by placing under anaerobic conditions for 6-24 hours prior to use.[1] An efficient and easy way to obtain suitable anaerobic conditions is through the use of **GasPak**™ EZ anaerobic systems or other alternative anaerobic systems.

Incubate immediately under anaerobic conditions or place in a holding jar flushed with oxygen-free gas(es) until sufficient plates are accumulated (but no longer than 3 hours).[2] Incubation should be at 35 ± 2°C for at least 48 hours.

Expected Results

After 48-72 hours of incubation, *Clostridium difficile* will appear as flat to low umbonate, yellow colonies with a ground glass-like appearance and a slightly filamentous edge. *C. difficile* colonies may be surrounded by a yellow zone of about 2-3 mm, depending on colony size and incubation time. Growth may be examined with a long-wave UV light for yellow fluorescence within 1 hour of removal from the anaerobic atmosphere. After exposure to air, colonies may become nonviable, which is usually accompanied by reversal of the color change to pink and a loss of fluorescence. Since some facultative anaerobic organisms potentially could produce reactions similar to *C. difficile*, it is recommended that an aerobically incubated plate be utilized in order to confirm that the isolate is an obligate anaerobe.

Limitations of the Procedure

This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with growth from the primary plating medium. For identification, the organism must be in pure culture. Complete identification may be performed using Gram reaction, cellular morphology, sensitivity to oxygen, biochemical reactions, susceptibility to antimicrobial agents and gas liquid chromatographic analysis of metabolic products. Some species of clostridia (e.g., *butyricum, histolyticum, innocuum, sordellii* and *subterminale*) may grow on this medium and produce yellow colonies and fluorescence. Also, the isolation of *Clostridium difficile* should not be relied upon for etiologic diagnosis of pseudomembranous colitis.[3] Other tests, such as **ColorPAC**™ *C. difficile* Rapid Toxin A Test or other toxin assays, along with clinical observations should be used.[4]

References

1. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
2. Martin. 1971. Appl. Microbiol. *22*:1168.
3. George, Rolfe and Finegold. 1982. J. Clin. Microbiol. *15*:1049.
4. Baron. 1989. Clin. Microbiol. Newsl. *11*:118.

Availability

BBL™ Clostridium Difficile Selective Agar

Cat. No. 222228 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Coagulase Mannitol Agar

Intended Use

Coagulase Mannitol Agar is used for the differentiation of *Staphylococcus aureus* from other species based on coagulase production and mannitol utilization.

Summary and Explanation

Staphylococci, both coagulase-positive and coagulase-negative *Staphylococcus* species (CoNS), have major medical significance.[1] Coagulase-producing staphylococci (*S. aureus*) may be differentiated and presumptively identified with this medium based on production of coagulase and mannitol utilization.

Chapman introduced the first selective medium for isolating and differentiating staphylococcal species.[2] Several years later, Zebovitz et al. and Marwin introduced tellurite-glycine media designed to selectively isolate coagulase-positive staphylococcal species.[3,4]

Esber and Faulconer developed the formula used in this medium.[5] In contrast to the earlier media, this formulation was developed as a general-purpose medium for fastidious organisms that also permitted differentiation of pathogenic staphylococci from other bacteria.

Principles of the Procedure

Coagulase Mannitol Agar aids in the differentiation of staphylococci by indicating the presence of coagulase and the utilization of mannitol. Coagulase production is dependant on the presence of mannitol, a protein factor in the brain heart infusion and blood serum (plasma).[5] During utilization of the mannitol, the pH of the medium drops, causing the bromcresol purple indicator to change from purple to yellow and producing yellow zones around these colonies. An opaque area of coagulated plasma forms around the colonies of organisms that also produce coagulase.

User Quality Control

Identity Specifications

BBL™ Coagulase Mannitol Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain some minute to very small tan flecks.
Solution:	4.7% solution, soluble in purified water upon boiling. Solution is medium to dark, purple, clear to slightly hazy.
Prepared Appearance:	Medium to dark, purple, clear to slightly hazy.
Reaction of 4.7% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Coagulase Mannitol Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM*	ATCC™	INOCULUM CFU	MANNITOL UTILIZATION	COAGULASE REACTION
Enterobacter aerogenes	13048	10^3-10^4	Yellow colonies with or without weak yellow zone	No zone
Proteus vulgaris	8427	10^3-10^4	Negative	No zone
Staphylococcus aureus	13150	10^3-10^4	Yellow zone	Opaque zone
Staphylococcus epidermidis	12228	10^3-10^4	Negative	No zone

**Recovery of all cultures should be good.*

In contrast, a coagulase-negative species that does not utilize mannitol, such as *Staphylococcus epidermidis*, does not change the color of the medium and it remains clear. Other coagulase-negative species may utilize mannitol and produce a yellow zone around the colonies, but an opaque zone will not be produced.

Formula

BBL™ Coagulase Mannitol Agar

Approximate Formula* Per Liter		
Brain Heart Infusion	5.0	g
Pancreatic Digest of Casein	10.5	g
Papaic Digest of Soybean Meal	3.5	g
Sodium Chloride	3.5	g
D-Mannitol	10.0	g
Agar	14.5	g
Bromcresol Purple	0.02	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 47 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and add 7-15% pretested, undiluted rabbit coagulase plasma with EDTA. Mix gently and pour into plates, approximately 18 mL per plate.
5. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: The use of **BBL** Coagulase Plasma, Rabbit with EDTA, in place of citrated plasma, prevents false-positive coagulase reactions by citrate-utilizing microorganisms.

Procedure

Inoculate and incubate the plates in an inverted position (agar side up) at 35 ± 2°C, and examine for growth after 18-24 hours. Avoid prolonged incubation because it may cause the opaque zones surrounding coagulase-positive organisms to become clear.

Expected Results

After 18-24 hours of incubation, coagulase-positive organisms will produce opaque zones; coagulase-negative organisms will produce no opacity. Organisms that utilize mannitol produce yellow zones. *S. aureus* may be presumptively identified as those colonies with opaque, yellow zones around them.

Limitations of the Procedure

Some old or mutant strains of *S. aureus* may be weak coagulase producers or exhibit negative coagulase reactions and should be subcultured and retested if in doubt. *Escherichia coli* also uses mannitol and may be weakly coagulase-positive. Colonial morphology and a Gram stain should readily allow for differentiation from *S. aureus*.

References

1. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
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Availability

BBL™ Coagulase Mannitol Agar

Cat. No. 211116 Dehydrated – 500 g

Columbia Agars
Columbia Agar Base • Columbia Blood Agar Base Columbia Blood Agar Base EH • Columbia Agar with 5% Sheep Blood • Columbia Agar with Fildes Enrichment and Bacitracin

Intended Use

Columbia Agar Base, without or with the addition of 5% (or 10%) sheep blood, is a highly nutritious, general-purpose medium for the isolation and cultivation of nonfastidious and fastidious microorganisms from a variety of clinical and non-clinical materials.

Columbia Blood Agar Base EH (Enhanced Hemolysis) is used with blood in isolating and cultivating fastidious microorganisms.

Columbia Agar with Fildes Enrichment and Bacitracin is used in qualitative procedures for isolation and cultivation of *Haemophilus* species from clinical specimens.

Columbia Agar Base meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Ellner et al.,[4] in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar. The base achieves the more rapid and luxuriant growth obtained from casein hydrolysate media with the sharply defined hemolytic reactions, more typical colonial morphology and improved pigment production achieved with media containing infusion peptone.

Columbia Agar Base is utilized as the base for media containing blood and for selective media formulations in which various combinations of antimicrobial agents are used as additives.

Sheep blood allows detection of hemolytic reactions and supplies the X factor (heme) necessary for the growth of many bacterial species but lacks V factor (nicotinamide adenine dinucleotide), since it contains NADase which destroys the NAD. For this reason, *Haemophilus influenzae*, which requires both the X and V factors, will not grow on this medium. Fildes found that supplementing nutrient agar with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae*.[5,6] The inclusion of bacitracin makes

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Columbia Blood Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.4% solution, soluble in purified water upon boiling. Solution is light to medium amber, opalescent with fine precipitate.
Prepared Appearance:	Plain – Light to medium amber, slightly opalescent to opalescent with fine precipitate.
	With sheep blood – Cherry red, opaque, no hemolysis.
Reaction of 4.4% Solution at 25°C:	pH 7.3 ± 0.2

Identity Specifications

Difco™ Columbia Blood Agar Base EH

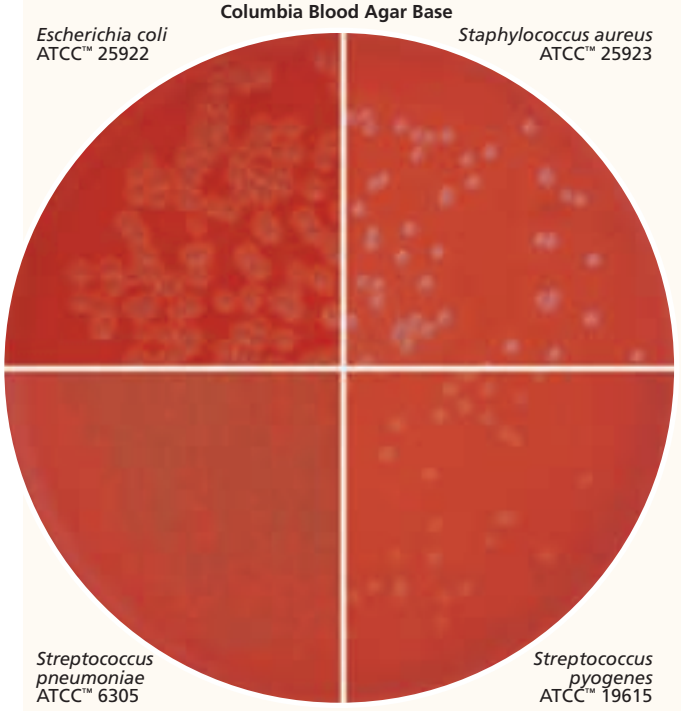
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent.
Prepared Appearance:	Plain – Light to medium amber, clear to slightly opalescent.
	With sheep blood – Medium to bright cherry red, opaque, no hemolysis.
Reaction of 3.9% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Columbia Blood Agar Base or Columbia Blood Agar Base EH

Prepare the medium per label directions without (plain) and with 5% sheep blood (SB) for Columbia Blood Agar Base and with 5% sheep blood for Columbia Blood Agar Base EH. Inoculate and incubate at 35 ± 2°C with 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB	HEMOLYSIS
Escherichia coli	25922	30-300	Good	Good	Beta
Neisseria meningitidis	13090	30-300	Good	Good	Gamma (none)
Staphylococcus aureus	25923	30-300	Good	Good	Beta
Streptococcus pneumoniae	6305	30-300	Good	Good	Alpha
Streptococcus pyogenes	19615	30-300	Good	Good	Beta



Continued

the enriched Columbia Agar medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from the upper respiratory tract.[7]

Columbia Agar with 5% sheep blood is a general all-purpose enriched primary isolation medium that allows growth of all clinically significant anaerobes and facultative anaerobes.[8,9] Columbia Agar supplemented with 5% sheep blood is recommended when processing clinical specimens for unusual organisms, such as *Bartonella bacilliformis*, the causative agent of Oroya fever and Peruvian wart.[8] Columbia Agar supplemented with 5% sheep blood and 20 µg of ampicillin per mL is used in isolating *Aeromonas* sp. from stool samples of patients showing clinical symptoms of gastroenteritis.[10]

Columbia Agar Base is used to prepare Modified Butzler Agar, which is a selective isolation medium for the detection of thermotolerant *Campylobacter* in food and animal feed.[11] Columbia Agar Base is a component of Oxford Medium and Columbia Blood Agar Base is a component of Modified Oxford Medium, both of which are used to detect *Listeria monocytogenes* in food and milk samples.[11-14] Columbia Agar is listed as one of the recommended media for the isolation of *Clostridia* sp. from nonsterile pharmaceutical products.[1]

C

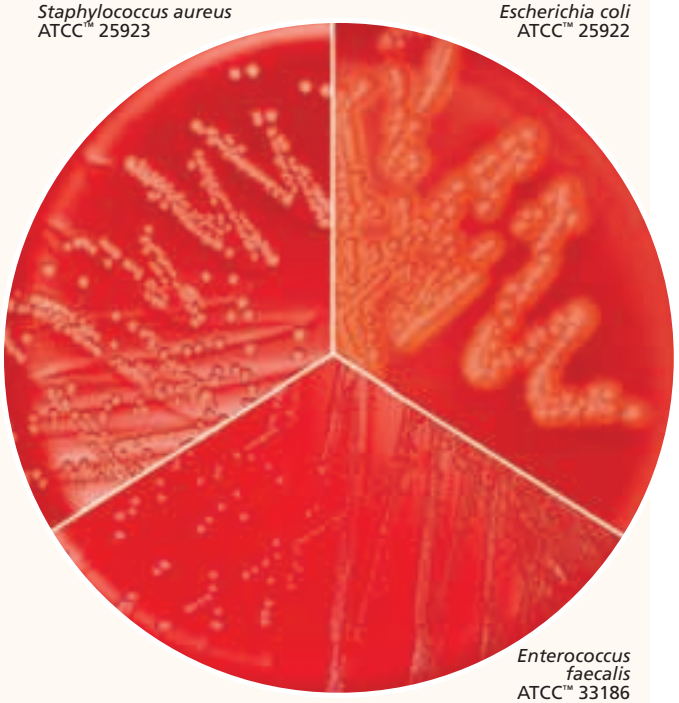
Identity Specifications

BBL™ Columbia Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.25% solution, soluble in purified water upon boiling. Solution is medium, yellow to tan, hazy.
Prepared Appearance:	Plain – Medium, yellow to tan, hazy. With sheep blood – Cherry red, opaque, no hemolysis.
Reaction of 4.25% Solution at 25°C:	pH 7.3 ± 0.2

BBL™ Columbia Agar (prepared)

Appearance:	Light to dark yellow and hazy with small cream particles in sediment; may appear as flocculation and contain small suspended insolubles.
Reaction at 25°C:	pH 7.3 ± 0.2



Cultural Response

BBL™ Columbia Agar Base

Prepare the medium per label directions without (plain) and with 5% sheep blood (SB). Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 48 hours (incubate *C. jejuni* at 42 ± 2°C for 48-72 hours). For *Clostridium sporogenes* (both strains), inoculate with fresh 24-48 hour Reinforced Clostridial Medium cultures, in duplicate, and incubate one set at 30-35°C and the other set at 35-37°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Campylobacter jejuni	33291	10^3	N/A	Good
Campylobacter jejuni	33292	10^3	N/A	Good
Candida albicans	10231	10^3-10^4	N/A	Good
Escherichia coli	25922	10^3-10^4	N/A	Good
Listeria monocytogenes	19115	10^3-10^4	N/A	Good
Pseudomonas aeruginosa	10145	10^3-10^4	Good	N/A
Shigella flexneri	12022	10^3-10^4	Good	N/A
Staphylococcus aureus	25923	10^3-10^4	Good	N/A
Streptococcus pneumoniae	6305	10^3-10^4	Good	N/A
Clostridium sporogenes	11437	<100	Growth (at 30-35°C)	N/A
Clostridium sporogenes	11437	<100	Growth (at 35-37°C)	N/A
Clostridium sporogenes	19404	<100	Growth (at 30-35°C)	N/A
Clostridium sporogenes	19404	<100	Growth (at 35-37°C)	N/A

BBL™ Columbia Agar (prepared)

Inoculate and incubate under anaerobic conditions at 30-35°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Clostridium sporogenes	11437	10-100	Growth
Clostridium sporogenes	19404	10-100	Growth

Principles of the Procedure

Columbia Agar Base supplemented with sheep, rabbit or horse blood derives its superior growth-supporting properties from the combination of peptones prepared from pancreatic digest of casein, meat peptic digest and heart pancreatic digest. Yeast extract and corn starch are also included in the formulation and serve as energy sources with yeast extract being a supplier of the B-complex vitamins. Sodium chloride maintains osmotic balance in the medium.

It should be noted that Columbia Sheep Blood Agar has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis.

Fildes enrichment is prepared by the action of the enzyme pepsin on defibrinated sheep blood. Bacitracin is a polypeptide antibiotic that is active mainly against gram-positive bacteria.

Formulae

Difco™ Columbia Blood Agar Base

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Proteose Peptone No. 3	5.0	g
Yeast Extract	5.0	g
Beef Heart, Infusion from 500 g	3.0	g
Corn Starch	1.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

BBL™ Columbia Agar Base

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Meat Peptic Digest	5.0	g
Yeast Extract	5.0	g
Heart Pancreatic Digest	3.0	g
Corn Starch	1.0	g
Sodium Chloride	5.0	g
Agar	13.5	g

Difco™ Columbia Blood Agar Base EH

Approximate Formula* Per Liter		
Pantone	12.0	g
Bitone H Plus	6.0	g
Enzymatic Digest of Animal Tissue	3.0	g
Starch	1.0	g
Sodium Chloride	5.0	g
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Columbia Blood Agar Base – 44 g;
   BBL™ Columbia Agar Base – 42.5 g;
   Difco™ Columbia Blood Agar Base EH – 39 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. For preparation of blood agar, cool the base to 45-50°C and add 5% sterile, defibrinated blood. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[8-10]

For food or milk samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[11-14]

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *Clostridium* sp. using Columbia Agar.[1]

Procedure

Refer to appropriate standard references for details on test methods to obtain isolated colonies from specimens or samples using Columbia Agar.[1,8-14] Incubate the plates at 35 ± 2°C for 18-72 hours under appropriate atmospheric conditions, or as instructed in the standard reference.[1,8-14]

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.

Expected Results

After the recommended incubation period, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

References

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Availability

Difco™ Columbia Blood Agar Base

AOAC BAM EP ISO

Cat. No.	279240	Dehydrated – 500 g
	279220	Dehydrated – 2 kg
	279230	Dehydrated – 10 kg

BBL™ Columbia Agar Base

AOAC COMPF EP ISO JP USP

Cat. No.	211124	Dehydrated – 500 g†
	211125	Dehydrated – 5 lb (2.3 kg)†
	211126	Dehydrated – 25 lb (11.3 kg)†
	215191	Prepared Plates – Pkg. of 20*†
	295661	Prepared Plates with Fildes Enrichment and Bacitracin – Pkg. of 20*

BBL™ Columbia Agar with 5% Sheep Blood

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	221165	Prepared Plates – Pkg. of 20*
	221263	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254005	Prepared Plates – Pkg. of 20*
	254071	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251165	Prepared Plates – Pkg. of 20*

Difco™ Columbia Blood Agar Base EH

Cat. No.	279030	Dehydrated – 500 g
	279010	Dehydrated – 2 kg
	279020	Dehydrated – 10 kg

BBL™ Fildes Enrichment

Cat. No.	211866	Prepared Tubes, 5 mL (K Tubes) – Pkg of 10*

**Store at 2-8°C.*
†QC testing performed according to USP/EP/JP performance specifications.

C

Columbia Anaerobe 5% Sheep Blood Agar

Intended Use

Columbia Anaerobe 5% Sheep Blood Agar is recommended for the general cultivation of anaerobes.

Summary and Explanation

Ellner[1], using Columbia Agar Base, formulated reducible anaerobic media designed to improve recovery of anaerobes with minimal difficulty. The reducing agents used were cysteine, palladium chloride and dithiothreitol. The presence of "organic" peroxides (or peroxide-like compounds) and the redox potential (Eh) of media are important factors in the determination of whether anaerobic organisms will grow in, or on, a particular medium. The addition of reducing agents to the medium reduces the inhibitory effects of the peroxides.

Principles of the Procedure

Columbia Anaerobe Sheep Blood Agar is a highly nutritious medium due to its content of peptones, yeast extract, beef extract, hemin, vitamin K_1 and sheep blood. The peptones provide nitrogenous growth factors, carbon, sulfur and trace ingredients. Yeast extract is an important source of B vitamins. Sodium chloride maintains osmotic equilibrium. Sheep blood constituents, hemin and vitamin K_1 provide growth factors required by certain obligate anaerobes.[2-5] The addition of L-cysteine HCl and dithiothreitol facilitates the lowering of the redox potential of medium.

Procedure

This medium should be reduced at room temperature immediately prior to inoculation by placing under anaerobic conditions for 18-24 hours.[4]

Use standard procedures to obtain isolated colonies from specimens. Inoculate an enrichment broth, such as BBL™ Enriched Thioglycollate Medium, at the same time as the primary plates to detect small numbers of anaerobes.

Incubate the plates and tubes immediately after inoculation, with plates in an inverted position (agar side up), under anaerobic conditions at 35°C, or place the media in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates and tubes is accumulated (no longer than 3 hours).[6] An efficient and easy way to obtain suitable anaerobic conditions is through the use of a BD GasPak™ EZ anaerobic system or an alternative anaerobic system. Incubate for at least 48 hours and, if no growth occurs, continue incubation for up to 7 days.

Expected Results

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Growth in liquid media is indicated by the presence of turbidity compared with uninoculated control.

Examine colonies using a dissecting microscope and with a long-wave UV lamp to detect fluorescence. Colonies of the pigmenting *Porphyromonas-Prevotella* species should fluoresce orange to brick red under long-wave UV light. Fluorescence is visible before pigmentation.

In order to determine the relationship to oxygen of each colony type present on the medium, follow established procedures.[7] Those colony types that prove to contain obligate anaerobes can be further studied using appropriate identification methods.

References

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5. Isenberg, Schoenknecht and von Graeventiz. 1979. Cumitech 9, Collection and processing of bacteriological specimens. Coord. ed., Rubin. American Society for Microbiology, Washington, D.C.
6. Martin. 1971. Appl. Microbiol. 22:1168.
7. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Columbia Anaerobe 5% Sheep Blood Agar

United States and Canada
Cat. No. 221928 Prepared Plates – Pkg. of 20*
221929 Prepared Plates – Ctn. of 100*

Japan
Cat. No. 251974 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Columbia Broth

Intended Use

Columbia Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

Columbia Broth is prepared according to the formulation described by Morello and Ellner.[1] In their study Columbia Broth, a medium developed for blood cultures, was superior to a commonly used general purpose broth for faster growth of *Staphylococcus aureus, Escherichia coli* and streptococci (viridans and enterococcus groups). Columbia Broth, in the presence of CO_2 and supplemented with SPS, is an excellent blood culture medium.[2] In the study by Morello and Ellner,[1] the addition of sodium polyanetholsulfonate (SPS) in Columbia Broth was emphasized. SPS is an anticoagulant that inhibits serum bactericidal activity against many bacteria, inhibits phagocytosis, inactivates complement, and neutralizes lysozymes and the aminoglycoside class of antibiotics.[2]

Principles of the Procedure

Peptones and yeast extract provide nitrogen, carbon, vitamins and trace nutrients essential for growth. Dextrose is added to the formula as a carbon energy source. The medium is buffered with Tris. Corn starch is omitted to reduce opalescence.[1] Cysteine is the reducing agent. Magnesium and iron are added to facilitate organism growth.

Formula

Difco™ Columbia Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Yeast Extract	5.0	g
Proteose Peptone No. 3	5.0	g
Tryptic Digest of Beef Heart	3.0	g
L-Cysteine HCl	0.1	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Magnesium Sulfate (anhydrous)	0.1	g
Ferrous Sulfate	0.02	g
Sodium Carbonate	0.6	g
Tris (Hydroxymethyl) Aminomethane	0.83	g
Tris (Hydroxymethyl) Aminomethane HCl	2.86	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Columbia Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon warming. Solution is light amber, clear to very slightly opalescent, may have a slight amount of fine precipitate.
Prepared Appearance:	Light amber, clear to very slightly opalescent, may have a slight amount of fine precipitate.
Reaction of 3.5% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Difco™ Columbia Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under appropriate conditions for 18-48 hours. Incubate *Bacteroides fragilis* anaerobically.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	25285	10^2-10^3	Good
Neisseria meningitidis	13090	10^2-10^3	Good
Pseudomonas aeruginosa	27853	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

Directions for Preparation from Dehydrated Product

1. Suspend 35 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. OPTIONAL: Sodium polyanetholesulfonate (SPS) may be added at this time with agitation to ensure a uniform solution. The culture medium should contain 0.025 to 0.05% SPS.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Process clinical specimens from different body sites as described in *Clinical Microbiology Procedures Handbook*,[2] *Manual of Clinical Microbiology*[3] or according to laboratory procedures.

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. *Neisseria* spp. may be inhibited by SPS in Columbia Broth. The addition of 1.2% gelatin may counteract the inhibitory effect, but SPS may also inhibit other organisms.[2]
2. Opalescence in Columbia Broth cannot always be relied upon as evidence of bacterial growth in the bottle.
3. It is possible for significant numbers of viable bacteria to be present in an inoculated and incubated blood culture bottle without the usual signs of bacterial growth.

References

1. Morello and Ellner. 1969. Appl. Microbiol. *17*:68.
2. Isenberg and Garcia (ed). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Columbia Broth

Cat. No. 294420 Dehydrated – 500 g

C

Columbia CNA Agar • Columbia CNA Agar, Modified Columbia PNA Agar

Intended Use

Columbia CNA Agar, Columbia CNA Agar, Modified, and Columbia PNA Agar, all supplemented with 5% sheep blood, are selective and differential media used for the isolation and differentiation of gram-positive microorganisms from clinical and nonclinical materials.

Summary and Explanation

Ellner et. al., in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar.[1] The Columbia Agar base, which achieves rapid and luxuriant growth and sharply defined hemolytic reactions, is utilized as the base for media containing blood and for selective formulations in which various combinations of antimicrobial agents are used as additives.

Ellner and his colleagues found that a medium consisting of 10 mg of colistin and 15 mg of nalidixic acid per liter in a Columbia Agar Base enriched with 5% sheep blood would support the growth of staphylococci, hemolytic streptococci and enterococci while inhibiting the growth of *Proteus*, *Klebsiella* and *Pseudomonas* species. In BBL™ Columbia CNA Agar with 5% Sheep Blood, the concentration of nalidixic acid has been reduced to 10 mg/L to increase the recovery of gram-positive cocci from clinical specimens. The concentration of nalidixic acid has been further reduced in Columbia CNA Agar, Modified to 5 mg/L.

In the Columbia PNA version of Ellner's medium, polymyxin B has been substituted for colistin (10 mg). Although the antimicrobial properties of the two agents are nearly the same, some species of gram-negative bacteria are more sensitive to polymyxin B than colistin.[2]

User Quality Control

Identity Specifications

BBL™ Columbia CNA Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.25% solution, soluble in purified water upon boiling. Solution is medium, tan to yellow, hazy.
Prepared Appearance:	Tan to yellow, hazy.
Reaction of 4.25% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Columbia CNA Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C with 3-5% CO_2 for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
Proteus mirabilis	12453	10^4-10^5	Partial to complete inhibition	–
Staphylococcus aureus	25923	10^3-10^4	Good	Beta
Streptococcus pneumoniae	6305	10^3-10^4	Good	Alpha
Streptococcus pyogenes	19615	10^3-10^4	Good	Beta, slight greening may be present

Enterococcus faecalis
ATCC™ 29212

Principles of the Procedure

These media derive their superior growth-supporting properties from the combination of peptones prepared from pancreatic digest of casein, peptic digest of animal tissue and beef extract. Yeast extract and corn starch are also included in the formulation and serve as energy sources, with yeast extract being a supplier of the B-complex vitamins.

Sheep blood supports the growth of fastidious organisms and allows detection of hemolytic reactions. It should be noted that this medium has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis.

The addition of the antimicrobial agents, colistin (or polymyxin B) and nalidixic acid, renders the medium selective for gram-positive microorganisms.[3] Colistin and polymyxin B disrupt the cell membrane of gram-negative organisms, whereas the nalidixic acid blocks DNA replication in susceptible gram-negative bacteria.[4]

Formula

BBL™ Columbia CNA Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	12.0	g
Peptic Digest of Animal Tissue	5.0	g
Yeast Extract	3.0	g
Beef Extract	3.0	g
Corn Starch	1.0	g
Sodium Chloride	5.0	g
Agar	13.5	g
Colistin	10.0	mg
Nalidixic Acid	10.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 42.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 12 minutes. Cool to 45-50°C.
4. Add 5% sterile, defibrinated sheep blood.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 35 ± 2°C for 24-48 hours in an aerobic atmosphere supplemented with carbon dioxide.

Expected Results

Typical colonial morphology on Colombia CNA Agar with 5% Sheep Blood is as follows:

Streptococci (non-group) D	Small, white to grayish. Beta or alpha hemolysis.
Enterococci (group D)	Small, but larger than group A streptococci, blue-gray. Beta or alpha hemolysis.
Staphylococci	Large, white to gray or cream to yellow, with or without hemolysis.
Micrococci	Large, white to gray or yellow to orange, with or without hemolysis.
Corynebacteria	Small to large, white to gray or yellow, with or without hemolysis.
Candida	Small, white.
Listeria monocytogenes	Small to large, blue-gray, with beta hemolysis.
Gram-negative bacteria	No growth to trace growth.

References

1. Ellner, Stoessel, Drakeford and Vasi. 1966. Am. J. Clin. Pathol. *45*:502.
2. Garrod and O'Grady. 1971. *In* Antibiotics and chemotherapy, 3rd ed. Williams & Wilkins, Baltimore, Md.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Estevez. 1984. Lab. Med. *15*:258.

Availability

BBL™ Columbia CNA Agar

Cat. No.	212104	Dehydrated – 500 g
	294221	Dehydrated – 5 lb (2.3 kg)
	212249	Dehydrated – 25 lb (11.3 kg)

BBL™ Columbia CNA Agar with 5% Sheep Blood

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	221352	Prepared Plates – Pkg. of 20*
	221353	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254007	Prepared Plates – Pkg. of 20*
	254072	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251352	Prepared Plates – Pkg. of 20*

BBL™ Columbia CNA Agar with 5% Sheep Blood// MacConkey II Agar

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	221600	Prepared **I Plate**™ Dishes – Pkg. of 20*
	221601	Prepared **I Plate**™ Dishes – Ctn. of 100*

Japan

Cat. No.	251600	Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ Columbia CNA Agar, Modified, with Sheep Blood// Enterococcosel™ Agar

Cat. No.	297413	Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ Columbia CNA Agar with 5% Sheep Blood// Levine EMB Agar

Cat. No.	295618	Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ Columbia CNA Agar with 5% Sheep Blood// EMB Agar, Modified (Holt-Harris and Teague)

Cat. No.	221941	Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ Columbia PNA Agar with 5% Sheep Blood// MacConkey II Agar

Cat. No.	297272	Prepared **I Plate**™ Dishes – Ctn. of 100*

**Store at 2-8°C.*

Cooke Rose Bengal Agar Antimicrobic Vial A

Intended Use

Cooke Rose Bengal Agar is used with or without Antimicrobic Vial A in isolating fungi from environmental and food specimens.

Antimicrobic Vial A is used in preparing microbiological culture media.

Summary and Explanation

Cooke Rose Bengal Agar is a selective medium for the isolation of fungi prepared according to the formula of Cooke.[1,2] Selectivity of the medium is increased by the addition of antibiotics.

A variety of materials and methods have been used to inhibit bacteria in an attempt to isolate fungi from mixed flora. Fungi are extremely successful organisms, as evidenced by their ubiquity in nature.[3] Waksman[4] described an acid medium consisting of peptone, dextrose, inorganic salts and agar for the isolation of fungi from soil. Cooke[1] used the Waksman[4] medium without adjustment to investigate the isolation of fungi from sewage. It was discovered that soy peptone was particularly suitable for use in this medium and that the combination of chlortetracycline, or oxytetracycline, with rose bengal increased the selectivity of the medium.

Antimicrobic Vial A contains sterile, desiccated chlortetracycline. It was originally used in preparing DTM Agar described by Taplin, Azias, Rebell and Blank[5] for the isolation of dermatophytes. Antimicrobic Vial A is applicable for use in various media requiring this antibiotic. Cooke[1] preferred chlortetracycline in Cooke Rose Bengal Agar due to the increased stability of the antibiotic.

Principles of the Procedure

Peptone provides nitrogen, carbon and vitamins in Cooke Rose Bengal Agar. Dextrose is an energy source. Rose bengal and chlortetracycline selectively inhibit bacterial growth and restrict the size and height of colonies of more rapidly growing molds. Monopotassium phosphate provides buffering capability. Magnesium sulfate is a source of divalent cations. Agar is the solidifying agent.

Formulae

Difco™ Cooke Rose Bengal Agar

Approximate Formula* Per Liter

Soy Peptone	5.0	g
Dextrose	10.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Agar	20.0	g
Rose Bengal	35.0	mg

Difco™ Antimicrobic Vial A

Contains 25 mg sterile desiccated chlortetracycline per 10 mL vial.

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Cooke Rose Bengal Agar

Dehydrated Appearance:	Pinkish-tan, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is pinkish red, slightly opalescent.
Prepared Appearance:	Deep pink, slightly opalescent.
Reaction of 3.6% Solution at 25°C:	pH 6.0 ± 0.2

Difco™ Antimicrobic Vial A

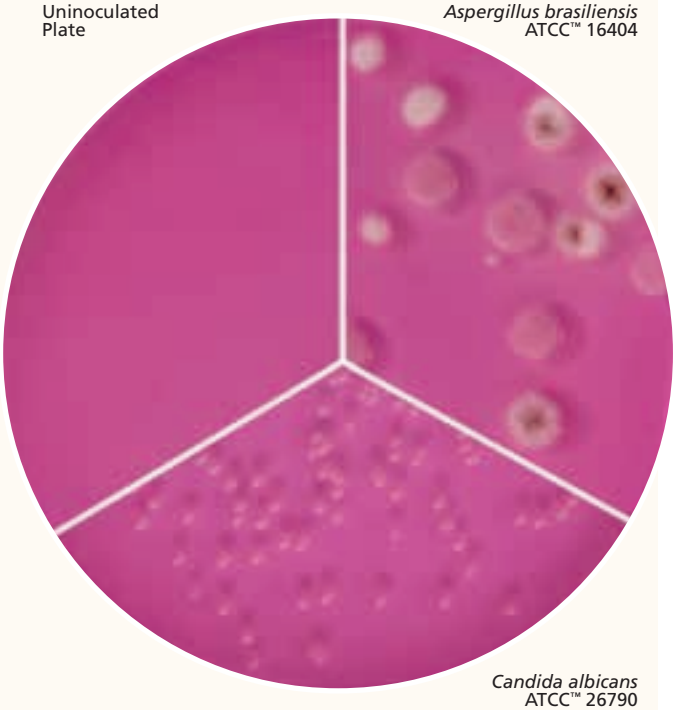
Desiccated Appearance:	Yellow cake or powder.
Rehydrated Appearance:	Yellow, clear solution.
Solution:	Soluble in 10 mL purified water.

Cultural Response

Difco™ Cooke Rose Bengal Agar and Antimicrobic Vial A

Prepare the medium with 35 µg per mL chlortetracycline (Antimicrobic Vial A added aseptically) per label directions. Inoculate and incubate at 25-30°C for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	Undiluted	Good
Candida albicans	26790	30-300	Good
Escherichia coli	25922	10^3	Inhibition
Saccharomyces cerevisae	9763	30-300	Good



Directions for Preparation from Dehydrated Product

Difco™ Cooke Rose Bengal Agar

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45°C.
4. Aseptically add 14 mL of rehydrated Antimicrobic Vial A (final concentration of 35 µg chlortetracycline per mL) or appropriate amount of another antibiotic.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Antimicrobic Vial A

1. Aseptically add 10 mL sterile purified water per vial of supplement.
2. Agitate gently to dissolve completely.
3. The resulting concentration of the rehydrated solution is 2.5 mg chlortetracycline per mL.

Procedure

Refer to appropriate references for specific procedures on the isolation and cultivation of fungi.

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Although this medium is selective primarily for fungi, microscopic examination is recommended for presumptive identification. Biochemical testing using pure cultures is required for complete identification.
2. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the complete medium; similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
3. Care should be taken not to expose this medium to light, since photo-degradation of rose bengal yields compounds that are toxic to fungi.[6,7]

References

1. Cooke. 1954. Antibiot. Chemother. *4*:657.
2. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed, online. American Public Health Association, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Waksman. 1922. J. Bacteriol. 7:339.
5. Taplin, Azias, Rebell and Blank. 1969. Arch. Dermatol. 99:203.
6. Banks, Board and Paton. 1985. Lett. Appl. Microbiol. *1*:7.
7. Beuchat and Cousin. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Cooke Rose Bengal Agar

SMWW

Cat. No. 270310 Dehydrated – 500 g

Difco™ Antimicrobic Vial A

SMWW

Cat. No. 233331 Vial – 6 x 10 mL*

**Store at 2-8°C.*

Cooked Meat Medium • Cooked Meat Medium with Glucose, Hemin and Vitamin K_1

Intended Use

Cooked Meat Medium and the enriched medium are used for the cultivation of anaerobes, especially pathogenic clostridia.

Summary and Explanation

In 1916, Robertson developed a cooked meat medium for use in the cultivation of certain anaerobes isolated from wounds.[1] The present formulation for Cooked Meat Medium is a modification of Robertson's original formula.

Cooked Meat Medium is still widely used for the cultivation and maintenance of clostridia and for determining proteolytic activity of anaerobes. For example, the medium is recommended for use in the enumeration and identification of *Clostridium perfringens* from food.[2] It supports the growth of most sporeforming and nonsporeforming obligate anaerobes and may be used for a variety of purposes including the maintenance of stock cultures. The medium is also useful as an enrichment broth for cultivating anaerobes that may be present in small numbers in a population and as a subculture medium for determination of proteolysis (meat digestion) and spore formation by *Clostridium* species.

Cooked Meat Medium with Glucose, Hemin and Vitamin K_1, is also recommended as a subculture medium for anaerobic isolates to be examined by gas liquid chromatography.[3]

Principles of the Procedure

Cooked Meat Medium provides a favorable environment for the growth of anaerobes, since the muscle protein in the heart tissue granules is a source of amino acids and other nutrients. The muscle tissue also provides reducing substances, particularly glutathione, which permits the growth of strict anaerobes.[4] The sulfhydryl groups, which exert the reducing effect, are more available in denatured protein; therefore, the meat particles are cooked for use in the medium.

Cooked Meat Medium with Glucose, Hemin and Vitamin K_1 is supplemented with added glucose, yeast extract, hemin and vitamin K_1 to enhance the growth of anaerobic microorganisms.

Growth is indicated by turbidity and, with some organisms, by the presence of gas bubbles in the medium. Disintegration and blackening of the meat particles indicates proteolysis. Gram

User Quality Control

Identity Specifications

Difco™ Cooked Meat Medium

Dehydrated Appearance:	Brown, homogeneous pellets.
Solution:	12.5% solution, partially insoluble in purified water. Solution is medium amber, clear to very slightly opalescent supernatant fluid over insoluble pellets.
Prepared Appearance:	Medium amber, clear to very slightly opalescent supernatant fluid over insoluble pellets.
Reaction of 12.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Cooked Meat Medium

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides vulgatus	8482	10^2-10^3	Good
Clostridium novyi	7659	10^2-10^3	Good
Clostridium perfringens	12924	10^2-10^3	Good
Clostridium sporogenes	11437	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good

Uninoculated Tube

Clostridium sporogenes ATCC™11437

stains or spore stains should be made to determine the shape and location of spores.

Formula

Difco™ Cooked Meat Medium

Approximate Formula* Per Liter

Beef Heart (from 454 g)	98.0	g
Proteose Peptone	20.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 12.5 g of the particles in 100 mL purified water (1.25 g/10 mL).
2. Let stand until all particles are thoroughly wetted and form an even suspension.
3. Autoclave at 121°C for 15 minutes. Reduce pressure slowly and cool without agitation.
4. If not used within 24 hours, reheat (100°C) prior to use to drive off absorbed oxygen.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **GasPak**™ EZ anaerobic system or an alternative anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling with caps loosened and cooling with tightened caps to room temperature before inoculation. Organisms to be cultivated must first be isolated in pure culture in an appropriate medium.

Using a sterile inoculating loop or needle, transfer growth from a fresh subculture medium, inoculating heavily in the area of meat particles. Incubate the tubes at 35 ± 2°C under anaerobic conditions for up to 7 days. It is recommended that an indicator of anaerobiosis be used.

Expected Results

In the cultivation of clostridia, saccharolytic organisms usually produce acid and gas. Growth of proteolytic organisms is generally characterized by blackening and dissolution of the meat particles.

References

1. Robertson. 1916. J. Pathol. Bacteriol. *20*:327.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC, International, Gaithersburg, Md.
3. Holdeman, Cato and Moore. 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnical Institute and State University, Blacksburg, Va.
4. Willis. 1977. Anaerobic bacteriology: clinical and laboratory practice, 3rd ed. Butterworths, London, England.

Availability

Difco™ Cooked Meat Medium

AOAC BAM CCAM COMPF

Cat. No. 226730 Dehydrated – 500 g

BBL™ Cooked Meat Medium

AOAC BAM CCAM COMPF

Cat. No. 221507 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10
221508 Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100

BBL™ Cooked Meat Medium with Glucose, Hemin and Vitamin K_1

BS12 CMPH2 MCM9

Cat. No. 297809 Prepared Tubes, 10 mL (C Tubes) – Ctn. of 100
295982 Prepared Tubes, 9 mL (K Tubes) – Pkg. of 10
299455 Prepared Tubes, 9 mL (K Tubes) – Ctn. of 100

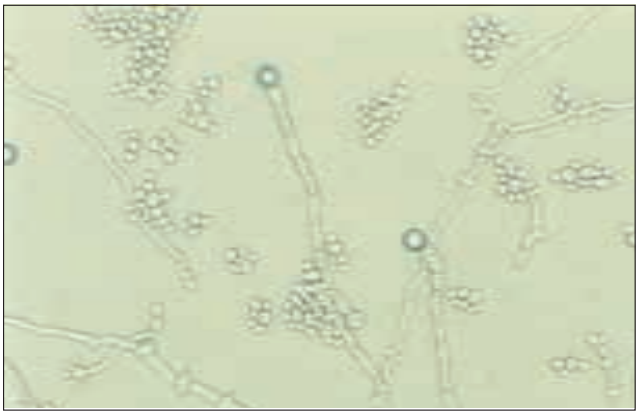
Corn Meal Agar • Corn Meal Agar with Polysorbate 80 • Corn Meal Agar with 1% Dextrose

Intended Use

Corn Meal Agar is a general-purpose medium for the cultivation of fungi. With the addition of polysorbate 80, it is utilized primarily for the testing of *Candida* species for their ability to produce chlamydospores. BBL™ prepared plates of Corn Meal Agar with Polysorbate 80 are deep-filled to reduce the effects of drying during prolonged incubation. Corn Meal Agar with 1% Dextrose enhances pigment production.

Summary and Explanation

Corn Meal Agar has been used for many years to cultivate fungi. Pollack and Benham reported on its usefulness for studying the morphology of *Candida*.[1] In 1960, Walker and Huppert modified the basic formulation of Corn Meal Agar by adding polysorbate 80, which stimulated rapid and abundant chlamydospore formation.[2] This modified formulation is recommended for the production and viualization of chlamydospores.[3]



Microscopic Photo of Chlamydospores

The addition of dextrose enhances fungal growth and pigment production.[4] Corn Meal Agar with Dextrose is commonly used in the differentiation of *Trichophyton* species based on chromogenesis.[5]

Principles of the Procedure

Corn Meal Agar is a relatively simple medium, consisting of an infusion of corn meal and agar. The infusion product contains sufficient nutrients to support the growth of fungal species. The polysorbate 80 is a mixture of oleic esters which, when added to the basal medium, stimulates the production of chlamydospores.[3] Dextrose is added to Corn Meal Agar to provide an energy source to enhance fungal growth and chromogenesis.

Formula

BBL™ Corn Meal Agar

Approximate Formula* Per Liter		
Corn Meal Infusion from (Solids)	2.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

BBL™ Corn Meal Agar

Dehydrated Appearance:	Coarse, homogeneous, free of extraneous material.
Solution:	1.7% solution, soluble in purified water upon boiling. Solution is pale to light, yellow to tan, slightly hazy to hazy.
Prepared Appearance:	Pale to light, yellow to tan, slightly hazy to hazy.
Reaction of 1.7% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

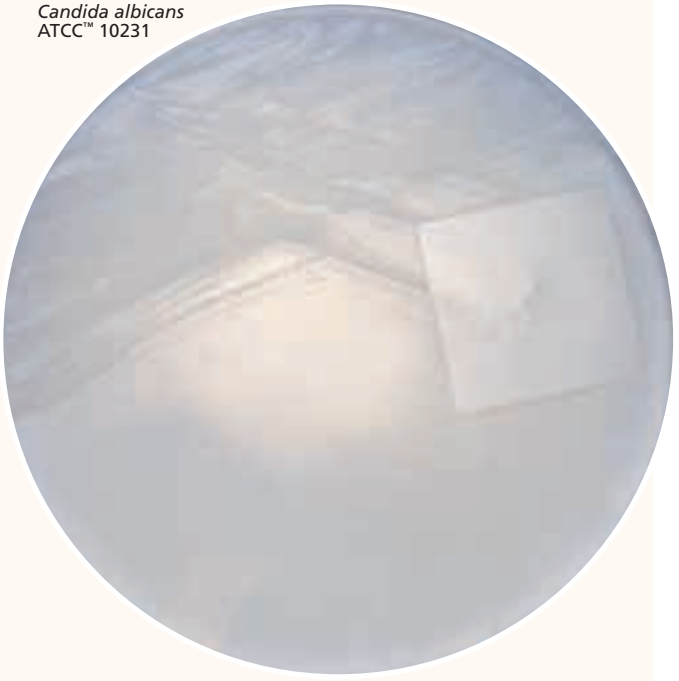
BBL™ Corn Meal Agar

Prepare the medium per label directions.Test for chlamydospore production. Using fresh cultures, streak two parallel lines approximately 1.5 cm long each and 1.0 cm apart. Make an S-shape by lightly streaking back and forth across the two parallel streak lines. Place a coverslip over the streak marks. Incubate at 25 ± 2°C for 4 days and examine microscopically.

ORGANISM	ATCC™	RECOVERY	CHLAMYDOSPORE PRODUCTION
Aspergillus brasiliensis (niger)	16404	Good	N/A
Candida albicans	10231	Good	Present
Candida albicans	60193	Good	Present
Candida kefyr	8553	Good	None

Corn Meal Agar with Polysorbate 80

Candida albicans
ATCC™ 10231



Directions for Preparation from Dehydrated Product

1. Suspend 17 g of the powder in 1 L of purified water. Add 1% polysorbate 80, or 1% dextrose, if desired. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To prepare plated media from agar deeps, place the agar deeps in a boiling water bath until the medium becomes liquefied (clear). Pour the molten medium into a sterile Petri dish and allow to solidify before use. Organisms to be cultivated for identification must first be isolated in pure culture on an appropriate medium.

Using an inoculating needle, streak the medium with growth from a pure culture and incubate at 25 ± 2°C. Examine at intervals for up to 28 days for growth and pigmentation.

Corn Meal Agar with 1% Dextrose should be incubated for up to 4 weeks to allow sufficient time for pigmentation to develop.

Test for the production of chlamydospores on medium containing polysorbate 80 using the Dalmau plate method.[6] With a sterile inoculating needle, lightly touch the yeast colony, and then make two separate streaks approximately 1.5 cm long each and 1.0 cm apart. Do not dig into the agar. Flame the needle, allow to cool. Then lightly make an S-shaped streak back and forth across the two original streak lines. Flame a coverslip and, after it cools, place it over the central area of the stab marks to provide slightly reduced oxygen tension.[3] Incubate the plates at room temperature (25 ± 2°C) for 24-48 hours. If the test is negative, reincubate plates an additional 48-72 hours and examine again.[7]

Expected Results

Observe cultures for growth and morphology. After 24-48 hours on medium containing polysorbate 80, most strains of *C. albicans* and *C. stellatoidea* will have formed typical chlamydospores.[3] Invert the plate and examine microscopically (low and high power objectives) for chlamydospore formation along the edge of the coverslip.

On Corn Meal Agar with 1% Dextrose, macroscopically observe chromogenesis.

Limitation of the Procedure

Corn Meal Agar with Dextrose is not recommended for detecting the production of chlamydospores by *Candida* species.

References

1. Pollack and Benham. 1960. J. Lab. Clin. Med. *50*:313.
2. Walker and Huppert. 1960. Tech. Bull. Reg. Med. Technol. *30*:10.
3. McGinnis. 1980. Laboratory handbook of medical mycology. Academic Press, New York, N.Y.
4. Conant, Smith, Baker and Callaway. 1971. Manual of clinical mycology, 3rd ed. W.B. Saunders Co., Philadelphia, Pa.
5. Haley and Callaway. 1978. Laboratory methods in medical mycology. HEW Publication No. (CDC) 78-8361. Center for Disease Control, Atlanta, Ga.
6. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
7. Campbell and Stewart. 1980. The medical mycology handbook. John Wiley & Sons, New York, N.Y.

Availability

BBL™ Corn Meal Agar

Cat. No.	211132	Dehydrated – 500 g
	297379	Prepared Pour Tubes, 20 mL – Pkg. of 10

BBL™ Corn Meal Agar with Polysorbate 80

B512

Cat. No.	221854	Prepared Plates (Deep Fill) – Pkg. of 10*
	297235	Prepared Pour Tubes, 20 mL – Pkg. of 10

BBL™ Corn Meal Agar with 1% Dextrose

Cat. No.	297229	Prepared Pour Tubes, 20 mL – Pkg. of 10

**Store at 2-8°C.*

Cystine Assay Medium

(See Amino Acid Assay Media)

Cystine Heart Agar

Intended Use

Cystine Heart Agar is used with hemoglobin for cultivating *Francisella tularensis* and without enrichment for cultivating gram-negative cocci and other microorganisms.

Summary and Explanation

Francisella tularensis was first described in humans in 1907.[1] Several media formulations were employed to isolate this microorganism. Initial formulations contained egg or serum and were difficult to prepare. Edward Francis,[2] who dedicated his career to the study of this organism, reported that blood dextrose cystine agar was a satisfactory medium for cultivating this fastidious pathogen. Shaw[3] added 0.05% cystine and 1% dextrose to Heart Infusion Agar for the cultivation of *F. tularensis*.

While experimenting with Francis' blood dextrose cystine agar, Rhamy[4] added hemoglobin to Cystine Heart Agar to develop a satisfactory medium for growth of *F. tularensis*.

Cystine Heart Agar, also known as Cystine Glucose Blood agar, is the historical medium of choice for isolating *F. tularensis*.[2]

Principles of the Procedure

Infusions from beef heart, peptone and L-cystine provide nitrogen, vitamins and amino acids in Cystine Heart Agar. Dextrose is a carbon source. Sodium chloride maintains the osmotic balance and agar is the solidifying agent.

Enrichment with 2% hemoglobin provides additional growth factors. Without enrichment, Cystine Heart Agar supports excellent growth of gram-negative cocci and other pathogenic microorganisms. Rabbit blood and antimicrobial agents can be added to this medium.[5]

Formula

Difco™ Cystine Heart Agar

Approximate Formula* Per Liter		
Beef Heart, Infusion from 500 g	10.0	g
Proteose Peptone	10.0	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
L-Cystine	1.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Francisella tularensis is a Biosafety Level 2 pathogen that can be transmitted by aerosols or by penetration of unbroken skin.[5] Wearing of gowns, gloves and masks is advocated for laboratory staff handling suspected infectious material.[6]

Directions for Preparation from Dehydrated Product

Enriched Medium

1. Suspend 10.2 g of the powder in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50-60°C.
4. Add 100 mL sterile 2% hemoglobin solution and mix well. Use:
   - Hemoglobin Solution 2%; or,
   - Prepare a 2% hemoglobin solution as follows: Place 2 g of hemoglobin powder in a dry flask. Add 100 mL of cold purified water while agitating vigorously. Continue intermittent agitation for 10-15 minutes until solution is complete. Autoclave at 121°C for 15 minutes. Cool to 50-60°C.
5. Dispense into sterile Petri dishes or tubes.
6. Test samples of the finished product for performance using stable, typical control cultures.

Unenriched Medium

1. Suspend 51 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

User Quality Control

Identity Specifications

Difco™ Cystine Heart Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent, may have fine precipitate.
Prepared Appearance:	Plain – Light to medium amber, slightly opalescent, may have a fine precipitate.
	With Hemoglobin – Chocolate, opaque.
Reaction of 5.1% Solution at 25°C:	pH 6.8 ± 0.2

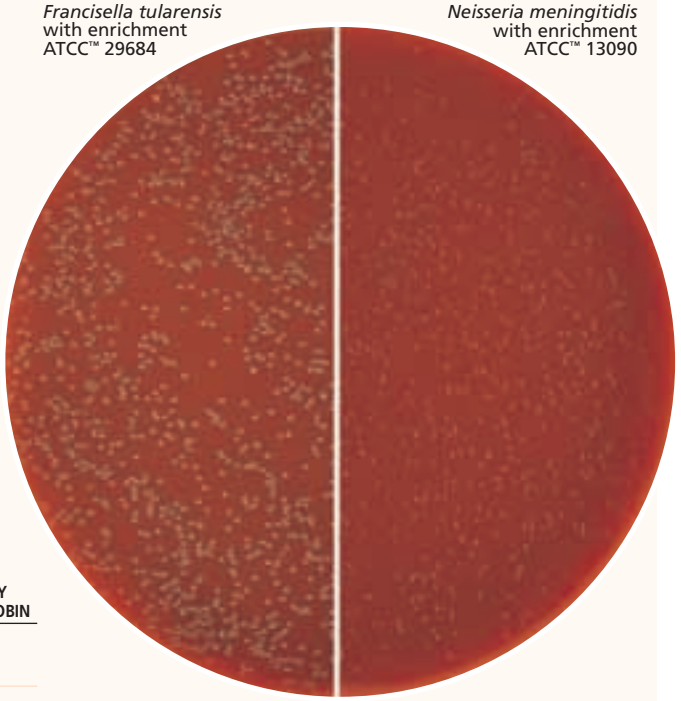
Cultural Response

Difco™ Cystine Heart Agar

Prepare the medium per label directions without and with hemoglobin. Incubate inoculated medium at 35 ± 2°C aerobically for 66-72 hours. Incubate *Neisseria meningitidis* under increased CO_2.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY W/O HEMOGLOBIN	RECOVERY W/HEMOGLOBIN
Francisella tularensis (BD 16223)*		10^2-10^3	N/A	Good
Neisseria meningitidis	13090	10^2-10^3	Good	Good
Staphylococcus aureus	25923	10^2-10^3	Good	Good
Streptococcus pneumoniae	6303	10^2-10^3	Good	Good

**Minimally, one strain of* F. tularensis *should be used for performance testing.* F. tularensis *ATCC 29684 can be substituted for BD Diagnostics strain 16223.*



3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Inoculate and streak specimens as soon as possible. For a complete discussion on the inoculation and identification of *Francisella*, consult appropriate references.
2. Overgrowth by contaminating organisms can be reduced by incorporating 100-500 units penicillin per mL into the medium.[1]

Expected Results

Refer to appropriate references and procedures for results.

References

1. Wong and Shapiro. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Francis. 1928. JAMA *91*:1155.
3. Shaw. 1930. Zentr. Bakt. I. Abt. Orig. *118*:216.
4. Rhamy. 1933. Am. J. Clin. Pathol. *3*:121.
5. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Difco™ Cystine Heart Agar

Cat. No. 247100 Dehydrated – 500 g

BBL™ Hemoglobin, Bovine, Freeze-Dried

Cat. No. 212392 Dehydrated – 500 g

BBL™ Hemoglobin Solution 2%

Cat. No. 211874 Bottle – 10 × 100 mL

C

Cystine Tryptic Agar

(See CTA Medium™)

Czapek-Dox Broth • Czapek Solution Agar

Intended Use

Czapek-Dox Broth and Czapek Solution Agar are used for cultivating fungi and bacteria capable of using inorganic nitrogen.

Summary and Explanation

Czapek-Dox Broth is a modification of the Czapek[1] and Dox[2] formula prepared according to Thom and Raper.[3] Czapek Solution Agar is prepared according to the formula given by Thom and Church.[4] The media are prepared with only inorganic sources of nitrogen and chemically defined sources of carbon. Czapek-Dox media are useful in a variety of microbiological procedures, including soil microbiology and fungi and mildew resistance tests. Thom and Raper[3] reported Czapek-Dox Broth and Czapek Solution Agar produce moderately vigorous growth of most saprophytic aspergilli and yield characteristic mycelia and conidia.

Czapek Solution Agar is recommended in *Standard Methods for the Examination of Water and Wastewater*[5] for the isolation of *Aspergillus*, *Penicillium, Paecilomyces* and related fungi.

Principles of the Procedure

Saccharose is the sole carbon source, and sodium nitrate is the sole nitrogen source in Czapek-Dox Broth and Czapek Solution Agar. Dipotassium phosphate is the buffering agent, and potassium chloride contains essential ions. Magnesium sulfate and ferrous sulfate are sources of cations. Agar is the solidifying agent in Czapek Solution Agar.

Formulae

Difco™ Czapek-Dox Broth

Approximate Formula* Per Liter		
Saccharose	30.0	g
Sodium Nitrate	3.0	g
Dipotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Potassium Chloride	0.5	g
Ferrous Sulfate	0.01	g

Difco™ Czapek Solution Agar

Approximate Formula* Per Liter		
Saccharose	30.0	g
Sodium Nitrate	2.0	g
Dipotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Potassium Chloride	0.5	g
Ferrous Sulfate	0.01	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Czapek-Dox Broth

1. Dissolve 35 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Czapek Solution Agar

1. Suspend 49 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Czapek-Dox Broth

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water. Solution is colorless, clear to very slightly opalescent and may have a slight precipitate.
Prepared Appearance:	Colorless, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 3.5% Solution at 25°C:	pH 7.3 ± 0.2

Difco™ Czapek Solution Agar

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	4.9% solution, soluble in purified water upon boiling. Solution is light amber, opalescent with a uniform flocculent precipitate.
Prepared Appearance:	Light amber, slightly opalescent, may have a slight precipitate.
Reaction of 4.9% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Czapek-Dox Broth

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 48-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus niger	9642	10^2-10^3	Good
Candida albicans	10231	10^2-10^3	Good
Candida tropicalis	750	10^2-10^3	Good
Saccharomyces cerevisiae	9763	10^2-10^3	Good

Difco™ Czapek Solution Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours (up to 72 hours if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus niger	9642	10^2-10^3	Good
Candida albicans	10231	10^2-10^3	Good
Penicillium rubrum	10520	10^2-10^3	Good
Streptococcus albus	3004	10^2-10^3	Good

Procedure

Refer to appropriate references for specific procedures for the cultivation of fungi and bacteria capable of utilizing inorganic nitrogen.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Czapek. 1902-1903. Beitr. Chem. Physiol. Pathol. *1*:540.
2. Dox. 1910. U.S. Dept. Agr. Bur. Anim. Ind. Bull. *120*:70.
3. Thom and Raper. 1945. Manual of the aspergilli. Williams & Wilkins Co., Baltimore, Md.
4. Thom and Church. 1926. The aspergilli. Williams & Wilkins Co., Baltimore, Md.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.

Availability

Difco™ Czapek-Dox Broth

Cat. No. 233810 Dehydrated – 500 g

Difco™ Czapek Solution Agar

SMWW

Cat. No. 233910 Dehydrated – 500 g

DCLS Agar

Intended Use

DCLS Agar (Desoxycholate Citrate Lactose Sucrose Agar) is a moderately selective culture medium for the isolation of *Salmonella* and *Shigella* from fecal specimens.

Summary and Explanation

DCLS Agar is a modification of Leifson's Desoxycholate Agar, a slightly selective and differential plating medium for enterics in which the degree of inhibition is accurately controlled by the substitution of pure chemicals for the largely undefined composition of bile.[1] DCLS Agar is only one of a number of modified desoxycholate-containing media and differs from the rest by its inclusion of sucrose.[2]

DCLS Agar supports good growth of cultures of *Shigella* and *Salmonella*, and inhibits the growth of coliforms and *Proteus*. In addition to the human pathogens, *S. pullorum* and *S. gallinarum* grow well.

Principles of the Procedure

This medium contains peptones and beef extract, which supply essential nutrients for the support of bacterial growth. The citrate and desoxycholate compounds serve as inhibitors of gram-positive bacteria and coliforms. The incorporation of two sugars permits the formation of red colonies by organisms that rapidly ferment either sucrose or lactose, or both; e.g., *Proteus vulgaris*, as well as typical coliforms. This permits the more accurate selection of members of the genera *Shigella* and *Salmonella*, which form colorless or nearly colorless colonies on DCLS Agar.

Formula

BBL™ DCLS Agar

Approximate Formula* Per Liter

Sodium Desoxycholate	2.5	g
Sodium Citrate	10.5	g
Lactose	5.0	g
Sucrose	5.0	g
Pancreatic Digest of Casein	3.5	g
Peptic Digest of Animal Tissue	3.5	g
Beef Extract	3.0	g
Sodium Thiosulfate	5.0	g
Neutral Red	0.03	g
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 50 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid excessive heating. DO NOT AUTOCLAVE.
3. Cool the medium to approximately 45°C and pour into plates using about 20 mL per plate. The plates may be used at once or refrigerated for a few days.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate and incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Expected Results

Typical colonial appearance on DCLS Agar is as follows:

Escherichia coli	Large, flat, pink to rose red with a zone of precipitated bile salts
Enterobacter/Klebsiella	Large, mucoid, pink
Proteus	Colorless to red
Salmonella	Colorless to pale pink
Shigella	Colorless to pale pink
Pseudomonas	Colorless to brown or green
Gram-positive bacteria	No growth

References

1. Leifson. 1935. J. Pathol. Bacteriol. *40*:581.
2. Hajna and Damon. 1956. Appl. Microbiol. *4*:341.

Availability

BBL™ DCLS Agar

Cat. No. 211144 Dehydrated – 500 g

Europe

Cat. No. 254012 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

User Quality Control

Identity Specifications

BBL™ DCLS Agar

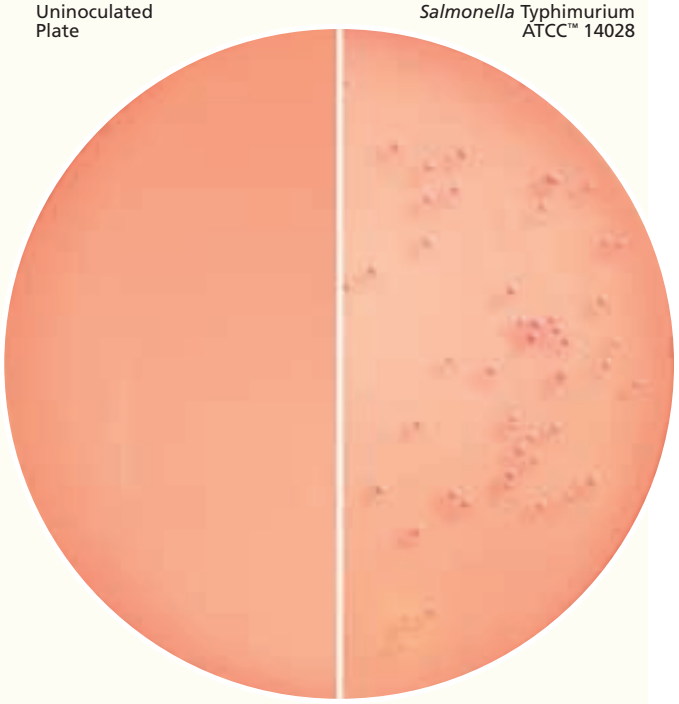
Dehydrated Appearance:	Fine, homogeneous powder.
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is medium to dark, red-orange to orange-rose, clear to slightly hazy.
Prepared Appearance:	Medium to dark, red-orange to orange-rose, clear to slightly hazy.
Reaction of 5.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ DCLS Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	10^4-10^5	Good	Pink to rose-red
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless to pale pink
Shigella flexneri	12022	10^3-10^4	Good	Colorless to pale pink
Enterococcus faecalis	29212	10^4-10^5	None	–



D/E Neutralizing Agar • D/E Neutralizing Broth

Intended Use

D/E (Dey/Engley) Neutralizing Agar has the ability to neutralize antimicrobial chemicals and is used for environmental sampling for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Prepared plates are provided for environmental monitoring. Sterile Pack and Isolator Pack **RODAC**™ prepared plates are particularly useful for monitoring surfaces in clean rooms and other environmentally-controlled areas and are also recommended for use in air sampling equipment such as the Surface Air System. **Finger Dab**™ Sterile Pack and Isolator Pack plates are intended for sampling gloved hands. **Hycheck**™ hygiene contact slides are used for assessing the microbiological contamination of surfaces and fluids.

D/E Neutralizing Broth is for the neutralization and testing of antiseptics and disinfectants according to the procedure of Engley and Dey.[1]

Summary and Explanation

Environmental contact sampling plates (**RODAC** plates) are specially constructed so that the D/E Neutralizing Agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden. These plates are used in a variety of programs to establish and monitor cleaning techniques and schedules.[2-5] After touching the surface to be sampled with the medium, the dish is covered and incubated at an appropriate temperature. The presence and number of microorganisms is determined by the appearance of colonies on the surface of the agar medium. Collection of samples from the same area before and after cleaning and treatment with a disinfectant permits the evaluation of the efficacy of sanitary procedures because of the neutralizing ability of the medium. The **RODAC** SL (Secure Lid) has three lugs on the base, providing a tight fit between lid and base to reduce accidental contamination.

The **Hycheck** hygiene contact slide is a double-sided paddle containing two agar surfaces for immersing into fluids or sampling surfaces. There are two slides containing D/E Neutralizing Agar: one slide contains D/E Neutralizing Agar on both sides; and another slide contains D/E Neutralizing Agar along with Tryptic Soy Agar.

D/E Neutralizing Broth is used for environmental sampling where neutralization of the chemical is important to determine its bactericidal or bacteriostatic activity. This medium will neutralize a broad spectrum of antiseptic and disinfectant chemicals, including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde.[1]

Principles of the Procedure

Peptone, yeast extract and dextrose are sources of nutrients required for the replication of microorganisms. The peptone provides nitrogenous compounds, including essential amino acids. Yeast extract is a rich source of B-complex vitamins. Dextrose is an energy source. Five neutralizers in this medium will inactivate a variety of disinfectant and antiseptic chemicals:

User Quality Control

Identity Specifications

Difco™ D/E Neutralizing Agar

Dehydrated Appearance:	Bluish-gray, homogeneous, appears moist and lumpy.
Solution:	5.4% solution, soluble in purified water upon boiling. Solution is lavender, opaque with a fine precipitate.
Prepared Appearance:	Lavender, opaque with a fine precipitate.
Reaction of 5.4% Solution at 25°C:	pH 7.6 ± 0.2

Difco™ D/E Neutralizing Broth

Dehydrated Appearance:	Bluish-gray, homogeneous, appears moist and lumpy.
Solution:	3.9% solution, soluble in purified water upon warming. Solution is purple, opaque with an even suspension of particles.
Prepared Appearance:	Purple, opaque with an even suspension of particles.
Reaction of 3.9% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Difco™ D/E Neutralizing Agar or D/E Neutralizing Broth

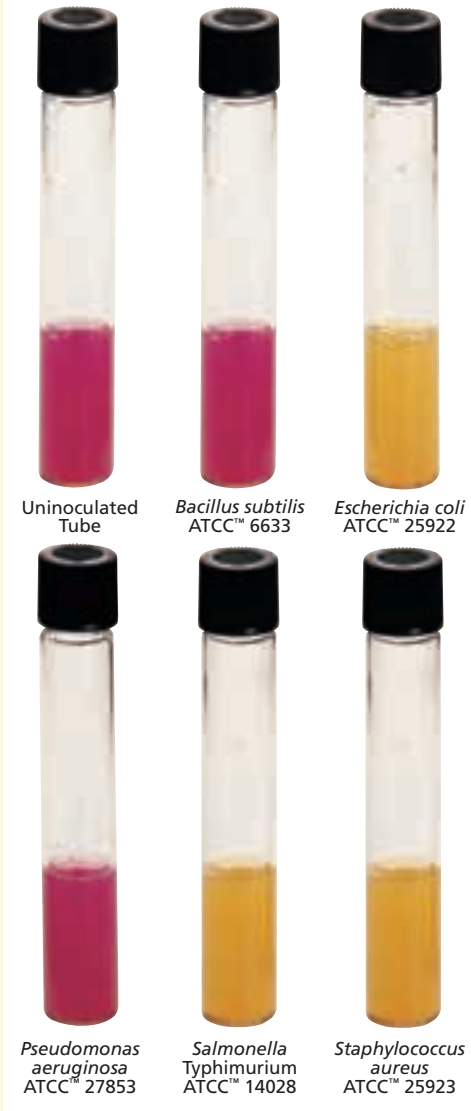
Prepare the medium per label directions. Inoculate plates and incubate at 35 ± 2°C for up to 40-48 hours. Prepare tubes with and without the addition of disinfectants; e.g., mercurials and quaternary ammonium compounds. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10^2-10^3	Good
Escherichia coli	25922	10^2-10^3	Good
Pseudomonas aeruginosa	27853	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good

Neutralization Test

Prepare D/E Neutralizing Agar per label directions. Inoculate 50 mL of D/E Neutralizing Agar with 0.1 mL of a heavy suspension of test organism and dispense into 150 ×15 mm Petri dishes of D/E Neutralizing Agar and Plate Count Agar. Place 1/2 inch sterile blank disks on each plate. Dispense 0.1 mL of each disinfectant solution onto two disks per medium. Incubate at 35 ± 2°C for 40-48 hours. D/E Neutralizing Agar should exhibit no zones of inhibition or zones significantly smaller than those found on Plate Count Agar.


Staphylococcus aureus
ATCC™ 25923



sodium bisulfite neutralizes aldehydes; sodium thioglycollate neutralizes mercurials; sodium thiosulfate neutralizes iodine and chlorine;[1] lecithin neutralizes quaternary ammonium compounds; and polysorbate 80, a non-ionic surface active agent, neutralizes substituted phenolics.[6-9] Bromcresol purple is incorporated as an indicator for dextrose utilization.

In the medium supplemented with penicillinase, the addition of penicillinase inactivates penicillinase-sensitive beta-lactam antibiotics.

In the prepared plated medium, the entire double-bagged (Sterile Pack) or triple-bagged (Isolator Pack) product is subjected to a sterilizing dose of gamma radiation so that the contents inside the outer bag are sterile.[10] This allows the inner bag(s) to be aseptically removed and brought into an environmentally-controlled area without introducing contaminants. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent the presence of environmental contaminants and not pre-existing microorganisms in the medium that may have been introduced during manufacture. The plate has a marked grid to facilitate counting organisms.

Due to the high concentration of lecithin in the broth medium (which renders the medium opaque), turbidity cannot be used to detect growth. Therefore, bromcresol purple and dextrose are added to the medium. Those organisms that ferment dextrose will turn the medium from purple to yellow. Growth of *Pseudomonas* species, which do not ferment dextrose, can be detected by the formation of a pellicle on the surface of the broth.[1]

Formulae

Difco™ D/E Neutralizing Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	5.0	g
Yeast Extract	2.5	g
Dextrose	10.0	g
Sodium Thioglycollate	1.0	g
Sodium Thiosulfate	6.0	g
Sodium Bisulfite	2.5	g
Polysorbate 80	5.0	g
Lecithin	7.0	g
Bromcresol Purple	0.02	g
Agar	15.0	g

Difco™ D/E Neutralizing Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ D/E Neutralizing Agar

1. Suspend 54 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ D/E Neutralizing Broth

1. Dissolve 39 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Agar

Selected surfaces are sampled by firmly pressing the agar medium against the test area. Hold the plates with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample. Do not move plate laterally; this spreads contaminants over the agar surface making resolution of colonies difficult. Slightly curved surfaces may be sampled with a rolling motion. Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.

Grid method:

1. Subdivide surface (floor or wall) into 36 equal squares per 100 square feet of area by striking five equidistant dividing lines from each of two adjacent sides.
2. These dividing lines intersect at twenty-five points.
3. Number these intersections consecutively in a serpentine configuration.
4. Use red numerals for odd numbers, black numerals for even numbers.
5. Omit number 13 which falls in the center of the total area.
6. Sample odd points at one sampling period, even points at the next sampling period.
7. For areas greater than 100 square feet, extend grid to include entire area.
8. For areas smaller than 25 square feet, divide the areas into twenty-five equal squares (sixteen intersections). Sample eight even-numbered or odd-numbered intersections at each sampling period.
9. For areas smaller than 25 and 100 square feet, divide into 36 equal squares as in #1.
10. Mark plates with intersection numbers.

Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days as required.

Broth

Add 1 mL of disinfectant solution to one tube of D/E Neutralizing Broth. Add culture as desired. Incubate tubes at 35°C. Examine for growth, indicated by a color change from purple to yellow or by pellicle formation.

To determine whether viable organisms are present in a "bacteriostatic" or "bactericidal" solution, inoculate samples from the broth onto D/E Neutralizing Agar or Standard Methods Agar plates. Incubate plates at 35-37°C for 48 hours.

Expected Results

Agar

After incubation, count visible colonies on plated medium. Counting of plates containing a profusion of growth can lead to considerable error. A basic decision to be made is whether distinct colony margins can be observed. Spreading colonies should be counted as one but care taken to observe other distinct colonies intermingled in the growth around the plate periphery or along a hair line. These should also be counted as one colony, as should bi-colored colonies and halo-type spreaders.

It is generally agreed that 200 colonies is the approximate maximum that can be counted on these plates. Colony counts may be recorded by:

1. Simply keeping individual counts.
2. Number of viable particles per square foot (agar area of RODAC™ plates is 3.97 square inches).
3. Means and standard deviations.

Subculture colonies of interest so that positive identification can be made by means of biochemical testing and/or microscopic examinations of organism smears.

Broth

If the disinfectant solution is bacteriostatic, it should be neutralized in the broth medium and the test organisms introduced into the broth will grow. Growth is indicated by a color change of the medium from purple to yellow, or pellicle formation.

Growth on the plates from negative broth tubes indicates a bacteriostatic substance. No growth on the plates from negative broth tubes indicates a bactericidal substance. All positive broth tubes should be positive on the plates.

References

1. Engley and Dey. 1970. Chem. Spec. Manuf. Assoc. Proc., Mid-Year Meet., p. 100.
2. Vesley and Michaelson. 1964. Health Lab. Sci. *1*:107.
3. Pryor and McDuff. 1969. Exec. Housekeeper, March.
4. Dell. 1979. Pharm. Technol. 3:47.
5. Wehr and Frank (ed.). 2004. Standard methods for the examinations of dairy products, 17th ed. American Public Health Association, Washington, D.C.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Quisno, Gibby and Foter. 1946. Am. J. Phar. *118*:320.
8. Erlandson and Lawrence. 1953. Science *118*:274.
9. Brummer. 1976. Appl. Environ. Microbiol. 32:80.
10. Association for the Advancement of Medical Instrumentation. 1984. Process control guidelines for gamma radiation sterilization of medical devices. AAMI, Arlington, Va.

Availability

Difco™ D/E Neutralizing Agar

COMPF SMD

Cat. No.	268620	Dehydrated – 500 g*
	268610	Dehydrated – 10 kg*

BBL™ D/E Neutralizing Agar

COMPF SMD

United States and Canada

Cat. No.	299969	Prepared Plates – Ctn. of 100*
	221232	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	222209	Sterile Pack **RODAC**™ Plates – Ctn. of 100*
	222243	Sterile Pack **RODAC**™ SL Plates – Pkg. of 10*
	222251	Sterile Pack **RODAC**™ SL Plates – Ctn. of 100*
	292645	Isolator Pack **RODAC**™ Plates – Pkg. of 10*
	292646	Isolator Pack **RODAC**™ Plates – Ctn. of 100*
	292647	Isolator Pack **Finger Dab**™ Plates – Pkg. of 10*

Europe

Cat. No.	257399	Sterile Pack **RODAC**™ Plates – Ctn. of 100*
	257398	Sterile Pack **RODAC**™ Plates (with penase) – Ctn. of 100*

Difco™ D/E Neutralizing Broth

AOAC BAM USDA

Cat. No.	281910	Dehydrated – 500 g*

BBL™ D/E Neutralizing Broth

AOAC BAM USDA

Cat. No.	298318	Prepared Tubes, 9 mL (A Tubes) – Ctn. of 100*

Difco™ Hycheck™ Hygiene Contact Slides

Cat. No.	290001	D/E Neutralizing Agar//D/E Neutralizing Agar– Box of 10 slides*
	290002	D/E Neutralizing Agar//Tryptic Soy Agar – Box of 10 slides*

**Store at 2-8°C.*

DNase Test Agars
DNase Test Agar • DNase Test Agar with Methyl Green
DNase Test Agar with Toluidine Blue

D

Intended Use

DNase Test Agar, DNase Test Agar with Methyl Green and DNase Test Agar with Toluidine Blue are differential media used for the detection of deoxyribonuclease activity to aid in the identification of bacteria isolated from clinical specimens.

Summary and Explanation

The DNase test is used to detect the degradation of deoxyribonucleic acid (DNA).[1,2] The test is useful for differentiating *Serratia* from *Enterobacter*, *Staphylococcus aureus* from coagulase-negative staphylococci, and *Moraxella catarrhalis* from *Neisseria* species.[1]

In 1957, Jeffries et al. described a rapid agar plate method for demonstrating DNase activity of microorganisms.[3] This procedure utilized a semi-synthetic medium with nucleic acid solution incorporated in the medium. Enzymatic activity is detected by flooding the plate with 1 N hydrochloric acid (HCl). A clear zone surrounding growth indicates a positive reaction.

DNase Test Agar is based on a medium developed by DiSalvo to adapt the rapid plate method for staphylococci.[4] Rather than using semi-synthetic medium, DiSalvo incorporated DNA into **Trypticase**™ Soy Agar and subsequently reported a correlation between coagulase production and DNase activity.

DNase Test Agar with Methyl Green contains a dye to eliminate the necessity of adding reagent to the agar plate following incubation.[5]

DNase Test Agar with Toluidine Blue contains a metachromatic dye to eliminate the necessity of reagent addition to the agar following incubation.[6] Toluidine blue may be toxic to some gram-positive cocci and, therefore, should be used primarily with *Enterobacteriaceae*.

Principles of the Procedure

Peptones provide amino acids and other complex nitrogenous substances to support bacterial growth. Sodium chloride maintains osmotic equilibrium. DNA is the substrate for DNase activity. DNase is an extracellular enzyme that breaks the DNA down into subunits composed of nucleotides.

The depolymerization of the DNA may be detected by flooding the surface of the medium with 1 N HCl and observing for clear zones in the medium surrounding growth. In the absence of DNase activity, the reagent reacts with the intact nucleic acid, resulting in the formation of a cloudy precipitate.

The HCl reagent is not needed to detect DNase activity on DNase Agar with Methyl Green. Methyl green forms a complex with intact (polymerized) DNA to form the green color of the medium. DNase activity depolymerizes the DNA, breaking down the methyl green-DNA complex, which results in the formation of colorless zones around colonies of the test organism. A negative test is indicated by the absence of a colorless zone around the colonies.

The HCl reagent is not needed to detect DNase activity on DNase Agar with Toluidine Blue. Toluidine blue forms a complex with intact (polymerized) DNA. In the intact DNA complex, the toluidine blue has the normal blue color. DNase activity depolymerizes the DNA, breaking down the dye-DNA complex. In the presence of nucleotides produced from the DNase depolymerization, the dye takes on its metachromatic color, forming pink to red zones around bacterial growth. A negative test is indicated when the medium remains blue.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ DNase Test Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 7.3 ± 0.2

Difco™ DNase Test Agar with Methyl Green

Dehydrated Appearance:	Light beige with slight green tint, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in purified water upon boiling. Solution is green, very slightly to slightly opalescent with slight precipitate.
Prepared Appearance:	Green, very slightly to slightly opalescent with slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ DNase Test Agar or DNase Test Agar with Methyl Green

Prepare the medium per label directions. Inoculate by streaking with a line of undiluted culture across the medium and incubate at 35 ± 2°C for up to 48 hours. For DNase Test Agar, flood the streak plates with 1N HCl and examine for clear zones around the streaks (positive reactions). For DNase Test Agar with Methyl Green, examine the streak plates for decolorized zones around the streaks (positive reactions).

ORGANISM	ATCC™	RECOVERY	REACTION
Serratia marcescens	8100	Good	+
Staphylococcus aureus	25923	Good	+
Staphylococcus epidermidis	12228	Good	–
Streptococcus pyogenes	19615	Good	+

Identity Specifications

BBL™ DNase Test Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.2% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 4.2% Solution at 25°C:	pH 7.3 ± 0.2

BBL™ DNase Test Agar with Toluidine Blue

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.2% solution, soluble in purified water upon boiling.Solution is medium to dark, blue, trace hazy to hazy.
Prepared Appearance:	Medium to dark, blue, trace hazy to hazy.
Reaction of 4.2% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ DNase Test Agar or DNase Test Agar with Toluidine Blue

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-24 hours. For DNase Test Agar, flood the plates with 1N HCl and examine for deoxyribonuclease activity. For DNase Test Agar with Toluidine Blue, examine for deoxyribonuclease activity.

ORGANISM	ATCC™	RECOVERY/ REACTION DNASE TEST AGAR	RECOVERY/REACTION DNASE TEST AGAR W/TOLUIDINE BLUE
Enterobacter aerogenes	13048	N/A	Good/–
Klebsiella pneumoniae	33495	Good/–	Good/–
Serratia marcescens	13880	Good/+	Good/+
Staphylococcus aureus	25923	Good/+	N/A
Staphylococcus epidermidis	12228	Good/–	N/A

Formulae

Difco™ DNase Test Agar

Approximate Formula* Per Liter

Tryptose	20.0	g
Deoxyribonucleic Acid	2.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

BBL™ DNase Test Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Papaic Digest of Soybean Meal	5.0	g
Deoxyribonucleic Acid	2.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

Difco™ DNase Test Agar with Methyl Green

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Proteose Peptone No. 3	10.0	g
Deoxyribonucleic Acid	2.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Methyl Green	0.05	g

BBL™ DNase Test Agar with Toluidine Blue

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Deoxyribonucleic Acid	2.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Toluidine Blue	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

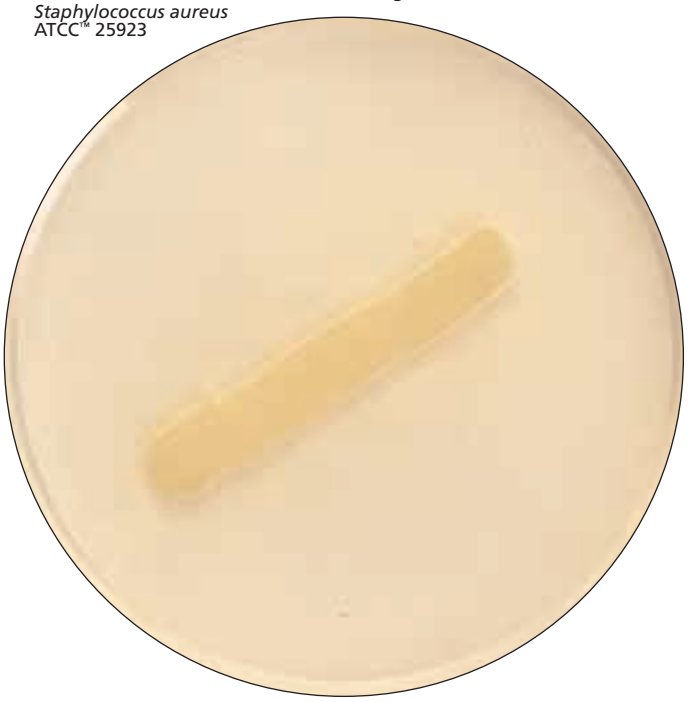
Directions for Preparation from Dehydrated Product

DNase Test Agar or DNase Test Agar with Methyl Green or DNase Test Agar with Toluidine Blue

1. Suspend 42 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

DNase Test Agar

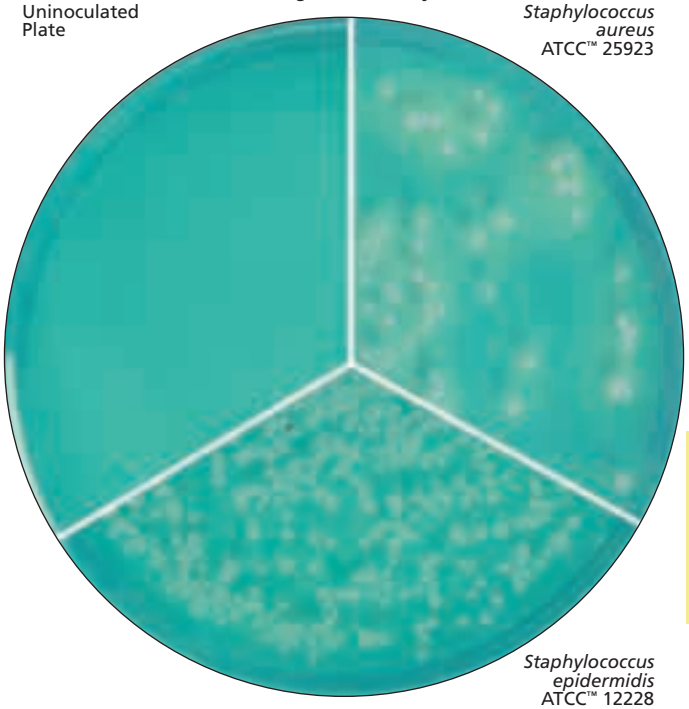
Staphylococcus aureus
ATCC™ 25923



DNase Test Agar with Methyl Green

Uninoculated Plate

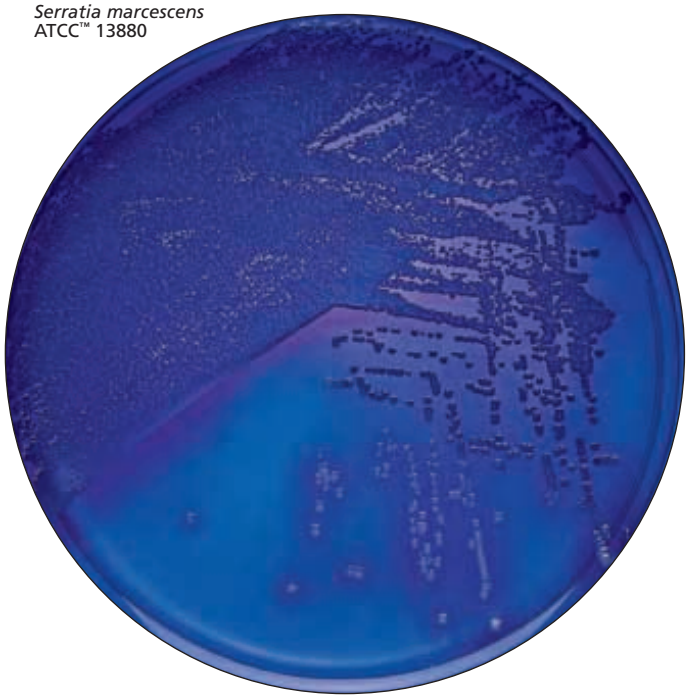
Staphylococcus aureus
ATCC™ 25923



Staphylococcus epidermidis
ATCC™ 12228

DNase Test Agar with Toluidine Blue

Serratia marcescens
ATCC™ 13880



3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate by making a single streak line using inoculum from an agar slant or plate. One plate may be inoculated with up to eight isolates by spot inoculation (1/8 to 1/4 inch) or streak inoculation (a single 1- to 2-inch line).

Incubate at 35 ± 2°C for 24-48 hours. Plates should be incubated in an inverted position. Incubate tubes with loosened caps.

Following incubation, flood DNase Test Agar plates with 1N HCl reagent and observe for reaction. Reagent addition is not required with DNase Test Agar with Methyl Green or with DNase Test Agar with Toluidine Blue.

Expected Results

A clear area surrounding growth (band/spot inocula) on DNase Test Agar after the addition of 1N HCl indicates a positive reaction, DNase activity. A negative reaction is indicated by no clearing and a cloudy precipitate around colonies and throughout medium due to precipitated salts in the medium.

A positive reaction on DNase Test Agar with Methyl Green is a distinct clear zone surrounding growth in an otherwise green-colored medium. The color of the medium remains unchanged if the test is negative.

On DNase Test Agar with Toluidine Blue, DNase activity is indicated by pink to red zones surrounding growth. The color of the medium remains unchanged if the test is negative.

References

1. Washington. 1985. Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Jeffries, Holtman and Guse. 1957. J. Bacteriol. *73*:590.
4. DiSalvo. 1958. Med. Tech. Bull. U.S. Armed Forces Med. J. *9*:191.
5. Schreier. 1969. Am. J. Clin. Pathol. *51*:711.
6. Smith, Hancock and Rhoden. 1969. Appl. Microbiol. *18*:991.

Availability

Difco™ DNase Test Agar

COMPF

Cat. No. 263220 Dehydrated – 500 g

BBL™ DNase Test Agar

COMPF

Cat. No. 211179 Dehydrated – 500 g

Europe
Cat. No. 255506 Prepared Plates – Pkg. of 20*

Mexico
Cat. No. 227450 Prepared Plates – Pkg. of 10*

Difco™ DNase Test Agar with Methyl Green

Cat. No. 222020 Dehydrated – 500 g

BBL™ DNase Test Agar with Methyl Green

United States and Canada
Cat. No. 297202 Prepared Plates – Pkg. of 20*

BBL™ DNase Test Agar with Toluidine Blue

BAM CCAM COMPF SMD

Cat. No. 299081 Dehydrated – 500 g

United States and Canada
Cat. No. 221856 Prepared Plates – Pkg. of 10*

Mexico
Cat. No. 211789 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

DRBC Agar

Intended Use

DRBC Agar is used for the enumeration of yeasts and molds.

Summary and Explanation

DRBC (Dichloran Rose Bengal Chloramphenicol) Agar is based on the Dichloran Rose Bengal Chlortetracycline Agar formula described by King, Hocking and Pitt.[1] DRBC Agar conforms with APHA guidelines for the mycological examination of foods, containing chloramphenicol rather than chlortetracycline as originally proposed.[2] DRBC Agar is a selective medium that supports good growth of yeasts and molds.

Principles of the Procedure

Peptone provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate is a buffering agent. Magnesium sulfate is a source of divalent cations and sulfate. The antifungal agent, dichloran, is added to the medium to reduce colony diameters of spreading fungi. The pH of the medium is reduced from 7.2 to 5.6 for improved inhibition of the spreading fungi.[1] The presence of rose bengal in the medium suppresses the growth of bacteria and restricts the size and height of colonies of the more rapidly growing molds. The concentration of rose bengal is reduced from 50 µg/mL to 25 µg/mL as found in Rose Bengal Chloramphenicol Agar for optimal performance with dichloran. Chloramphenicol is included in this medium to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing molds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species. In addition, rose bengal is taken up by yeast and mold colonies, which allows these colonies to be easily recognized and enumerated. Reduced recovery of yeasts may be encountered due to increased activity of rose bengal at pH 5.6.[1] Agar is the solidifying agent.

Formula

Difco™ DRBC Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	5.0	g
Dextrose	10.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Dichloran	2.0	mg
Rose Bengal	25.0	mg
Chloramphenicol	0.1	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 31.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure[2,3]

1. Inoculate 0.1 mL of appropriate decimal dilutions of the sample in duplicate onto the surface of DRBC Agar plates. The plates should be dried overnight at room temperature. Spread the inoculum over the entire surface of the plate using a sterile, bent-glass rod.
2. Incubate plates upright at 22-25°C. Examine for growth of yeasts and molds after 3, 4 and 5 days incubation.

Expected Results

Colonies of molds and yeasts should be apparent within 5 days of incubation. Colonies of yeast appear pink due to the uptake of rose bengal. Report the results as colony-forming units per gram or milliliter of sample.

User Quality Control

Identity Specifications

Difco™ DRBC Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	3.16% solution, soluble in purified water upon boiling.Solution is reddish pink, very slightly to slightly opalescent.
Prepared Appearance:	Bright pink, very slightly to slightly opalescent.
Reaction of 3.16% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Difco™ DRBC Agar

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for up to 5 days. For *A. niger*, spot inoculate.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus niger	1015	Undiluted	Good
Candida albicans	10231	10^2-10^3	Good
Escherichia coli	25922	10^3	None to poor
Micrococcus luteus	10240	10^3	None to poor

Aspergillus niger
ATCC™ 1015

D

Limitations of the Procedure

1. Although this medium is selective primarily for fungi, microscopic examination is recommended for presumptive identification. Biochemical testing using pure cultures is required for complete identification.
2. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the medium; similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
3. Care should be taken not to expose this medium to light, since photo-degradation of rose bengal yields compounds that are toxic to fungi.[2-4]

References

1. King, Hocking and Pitt. 1979. Appl. Environ. Microbiol. *37*:959.
2. Beuchat and Cousin. 2001. *In* Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association. Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Banks, Board and Paton. 1985. Lett. Appl. Microbiol. *1*:7.

Availability

Difco™ DRBC Agar

BAM CCAM COMPF SMD

Cat. No. 258710 Dehydrated – 500 g

Decarboxylase Differential Media
Decarboxylase Base Moeller • Decarboxylase Medium Base • Lysine Decarboxylase Broth • Moeller Decarboxylase Broth Base • Moeller Decarboxylase Broth with Arginine • Moeller Decarboxylase Broth with Lysine • Moeller Decarboxylase Broth with Ornithine

Intended Use

Decarboxylase media are used in the biochemical differentiation of gram-negative enteric bacilli based on the production of arginine dihydrolase and lysine and ornithine decarboxylase.

Decarboxylase Medium Base, with added arginine, lysine or ornithine is used for the same purpose.

Lysine Decarboxylase Broth is used for differentiating microorganisms based on lysine decarboxylation.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Decarboxylase Base Moeller

Dehydrated Appearance: Light to medium tan, free-flowing, homogeneous.

Solution: 1.05% solution, soluble in purified water upon boiling. Solution is yellowish-red, slightly opalescent.

Prepared Appearance: Yellowish-red, very slightly opalescent.

Reaction of 1.05% Solution at 25°C: pH 6.0 ± 0.2

Difco™ Decarboxylase Medium Base

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 0.9% solution, soluble in purified water upon warming. Solution is purple, clear.

Prepared Appearance: Purple, clear.

Reaction of 0.9% Solution at 25°C: pH 6.8 ± 0.2

Difco™ Lysine Decarboxylase Broth

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 1.4% solution, soluble in purified water upon boiling. Solution is purple, clear.

Prepared Appearance: Purple, clear.

Reaction of 1.4% Solution at 25°C: pH 6.8 ± 0.2

Cultural Response

Difco™ Decarboxylase Base Moeller

Prepare the medium per label directions with and without 1% L-lysine HCl. Inoculate tubes, overlaying with sterile mineral oil, and incubate at 35 ± 2°C for 18-48 hours. Purple color indicates a positive decarboxylase reaction; a yellow color is negative.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION WITHOUT LYSINE	REACTION WITH LYSINE
Escherichia coli	25922	10^3	Good	Yellow	Purple
Shigella flexneri	12022	10^3	Good	Yellow	Yellow

Difco™ Decarboxylase Medium Base

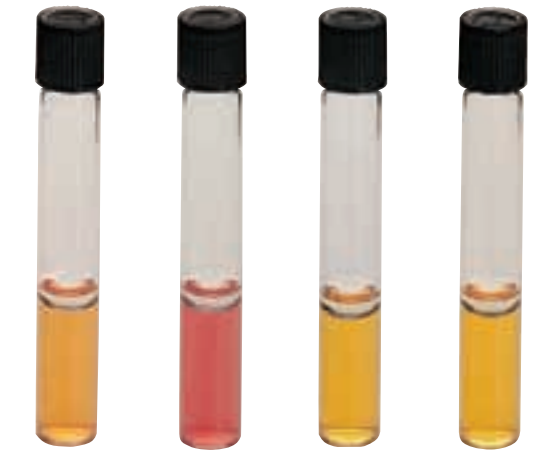
Prepare the medium per label directions. Inoculate tubes, overlaying with sterile mineral oil, and incubate at 35 ± 2°C for 40-48 hours. Purple color indicates a positive decarboxylase reaction; a yellow color is negative.

ORGANISM	ATCC™	INOCULUM CFU	REACTION WITH LYSINE	REACTION WITH ORNITHINE	REACTION WITH ARGININE
Proteus vulgaris	13315	10^3	–	–	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3	+	+	+

Difco™ Lysine Decarboxylase Broth

Prepare the medium per label directions. Inoculate tubes, overlaying with sterile mineral oil, and incubate at 35 ± 2°C for 18-48 hours. Purple color indicates a positive decarboxylase reaction; a yellow color is negative.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Escherichia coli	25922	10^3	Good	+
Proteus vulgaris	13315	10^3	Good	–



Escherichia coli ATCC™ 25922 | *E. coli* w/Lysine ATCC™ 25922 | *Shigella flexneri* ATCC™ 12022 | *S. flexneri* w/Lysine ATCC™ 12022

Decarboxylase Base Moeller

Uninoculated Tube | *Salmonella* Typhimurium ATCC™ 14028 | *Proteus vulgaris* ATCC™ 13315

Decarboxylase Medium Base

Escherichia coli ATCC™ 25922 | *Proteus vulgaris* ATCC™ 13315

Lysine Decarboxylase Broth

Continued

Identity Specifications

BBL™ Moeller Decarboxylase Broth Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.05% solution, soluble in purified water. Solution is light to medium, purple trace green to green tan purple, trace gray and rose acceptable, clear to slightly hazy.
Prepared Appearance:	Light to medium, purple trace green to green tan purple, trace gray and rose acceptable, clear to slightly hazy.
Reaction of 1.05% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

BBL™ Moeller Decarboxylase Broth Base

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C under appropriate atmospheric conditions for 4 days.

ORGANISM	ATCC™	REACTION WITHOUT LYSINE	REACTION WITH LYSINE
Enterobacter cloacae	13047	–	–
Klebsiella pneumoniae	33495	–	+

Summary and Explanation

Moeller introduced the decarboxylase media for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase.[1-3] These media are a useful adjunct to other biochemical tests for the speciation and identification of the *Enterobacteriaceae* and other gram-negative bacilli.[4-8] The production of ornithine decarboxylase is particularly useful for differentiating *Klebsiella* and *Enterobacter* species. *Klebsiella* species are non-motile and, except for *K. ornithinolytica*, do not produce ornithine decarboxylase, while most *Enterobacter* species are motile and, except for *E. agglomerans*, usually produce this enzyme.[6]

Falkow obtained valid and reliable results with a lysine decarboxylase medium he developed to differentiate and identify *Salmonella* and *Shigella*.[9] Although his modification of the Moeller formula was originally described as a lysine medium only, further study by Falkow and then by Ewing, Davis and Edwards,[10] substantiated the use of the medium for ornithine and arginine decarboxylase reactions as well.

Ewing, Davis and Edwards[10] compared the Falkow decarboxylase medium base to the Moeller medium and reported that, although the two methods compared favorably in most cases, the Moeller medium was found to be more reliable for cultures of *Klebsiella* and *Enterobacter*. They concluded that the Moeller method should be regarded as the standard or reference method, although the Falkow fomula is suitable for determining decarboxylase reactions for most members of the *Enterobacteriaceae* except for *Klebsiella* and *Enterobacter*. The Moeller medium is also particularly useful in the identification of *Aeromonas*, *Plesiomonas*, *Vibrio* spp. and nonfermentative gram-negative bacilli.[11]

Decarboxylase tests are important in the differentiation and identification of a wide variety of microorganisms and are outlined in numerous standard methods.[12-15]

Decarboxylase Base Moeller conforms with the Moeller formulation while Decarboxylase Medium Base is prepared according to the formula described by Falkow. Lysine Decarboxylase Broth is the Falkow medium with L-lysine added in 0.5% concentration.

Principles of the Procedure

Decarboxylase basal media consist of peptones and beef or yeast extract to supply the nitrogenous and other nutrients necessary to support bacterial growth. Pyridoxal is an enzyme co-factor for the amino acid decarboxylase. Dextrose is a fermentable carbohydrate. Bromcresol purple and cresol red are pH indicators. The amino acids lysine, ornithine or arginine are added to the basal medium at a concentration of 10.0 g/L to detect the production of the enzyme specific for these substrates.

When the medium is inoculated with a bacterium that is able to ferment dextrose, acids are produced that lower the pH of the medium and change the color of the indicator from purple to yellow. The acidic condition also stimulates decarboxylase activity. If the organism produces the appropriate enzyme, the amino acid in the medium is degraded, yielding a corresponding amine. Decarboxylation of lysine yields cadaverine, while decarboxylation of ornithine yields putrescine. Arginine is first hydrolyzed to form ornithine, which is then decarboxylated to form putrescine. The production of these amines elevates the pH of the medium, changing the color of the indicator from yellow to purple or violet. If the organism does not produce the appropriate enzyme, the medium remains acidic (yellow). Consult the reference for more information.[16]

Each isolate to be tested must also be inoculated into a tube of the basal medium that does not contain the amino acid. If this tube becomes alkaline, the test is invalid.

To obtain the appropriate reactions, the inoculated tubes must be protected from air with a layer of sterile mineral oil. Exposure to air may cause alkalinization at the surface of the medium, which could cause a decarboxylase-negative organism to appear positive.

Formulae

Difco™ Decarboxylase Base Moeller

Approximate Formula* Per Liter

Peptone	5.0 g
Beef Extract	5.0 g
Dextrose	0.5 g
Bromcresol Purple	0.01 g
Cresol Red	5.0 mg
Pyridoxal	5.0 mg

BBL™ Moeller Decarboxylase Broth Base

Approximate Formula* Per Liter

Peptic Digest of Animal Tissue	5.0 g
Beef Extract	5.0 g
Dextrose	0.5 g
Bromcresol Purple	0.01 g
Cresol Red	5.0 mg
Pyridoxal	5.0 mg

Difco™ Decarboxylase Medium Base

Approximate Formula* Per Liter		
Peptone	5.0	g
Yeast Extract	3.0	g
Dextrose	1.0	g
Bromcresol Purple	0.02	g

Difco™ Lysine Decarboxylase Broth

Approximate Formula* Per Liter		
Peptone	5.0	g
Yeast Extract	3.0	g
Dextrose	1.0	g
L-Lysine	5.0	g
Bromcresol Purple	0.02	g

** Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Decarboxylase Base Moeller

1. Suspend 10.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 10 g of L-amino acid or 20 g of DL-amino acid and dissolve. (When adding ornithine, adjust pH using approximately 4.6 mL 1N NaOH per liter.)
4. Autoclave at 121°C for 10 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Moeller Decarboxylase Broth Base

1. Suspend 10.5 g of the powder in 1 L of purified water. Add 1% of L-(or 2% of DL-)lysine, arginine or ornithine, as desired. Do not add the amino acid to the control broth.
2. Mix until a uniform suspension is obtained. Heat if necessary.
3. Autoclave at 121°C for 10 minutes. A small amount of flocular precipitate may be present in the ornithine broth, but it does not interfere with the reactions.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Decarboxylase Medium Base

1. Suspend 9 g of the powder in 1 L of purified water and warm to dissolve completely.
2. Add 5 g of L-amino acid or 10 g of DL-amino acid and warm to dissolve completely. Adjust pH if necessary.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Lysine Decarboxylase Broth

1. Suspend 14 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the broth media by transferring one or two colonies from the surface of a fresh culture with an inoculating loop or needle and mix to distribute the culture throughout the medium. Overlay the medium in each tube with 1 mL sterile mineral oil.

Incubate the tubes with caps tightened at 35 ± 2°C. Examine for growth and decarboxylase reactions after 18-24, 48, 72 and 96 hours before reporting as negative. The medium will become yellow initially, if the dextrose is fermented, and then will gradually turn purple if the decarboxylase or dihydrolase reaction occurs and elevates the pH.

Expected Results

Compare the color of tubes of media containing the specific amino acids with the color of control tubes of basal media (without amino acid) that have been inoculated with the same isolate. If inoculated control tubes show an alkaline reaction, the test is invalid; i.e., either improperly performed or the test organisms can degrade the peptone sufficiently to produce an alkaline reaction in the absence of a specific amino acid.

The medium becomes purple to violet if the reaction is positive (alkaline). A yellow color indicates a negative test; i.e., the organism does not produce the appropriate enzyme.

Limitations of the Procedure

1. If isolated or received on a selective medium, the organism should be subcultured to **Trypticase**™ Soy Agar with 5% Sheep Blood or other suitable culture medium before attempting to determine decarboxylase or dihydrolase activity.
2. Biochemical characteristics of the *Enterobacteriaceae* serve to confirm presumptive identification based on cultural, morphological, and/or serological findings. Therefore, biochemical testing should be attempted on pure culture isolates only and subsequent to differential determinations.
3. The decarboxylase reactions are part of a total biochemical profile for members of the *Enterobacteriaceae* and related organisms. Results obtained from these reactions, therefore, can be considered presumptively indicative of a given genus or species. However, conclusive and final identification of these organisms cannot be made solely on the basis of the decarboxylase reactions.
4. If layers of yellow and purple appear after incubation, shake the test tube gently before attempting to interpret results.
5. If a reaction is difficult to interpret, compare the tube in question to an uninoculated control tube. Any trace of purple after 24 hours of incubation is a positive test.
6. A gray color may indicate reduction of the indicator. Additional indicator may be added before the results are interpreted.[12]
7. *Salmonella gallinarum* gives a delayed positive ornithine decarboxylase reaction, requiring 5-6 days incubation.[3] Many strains of *E. coli*, including those that ferment adonitol, may exhibit a delayed reaction.[3]

8. Decarboxylase Medium Base is not satisfactory for the determination of lysine decarboxylase activity with the two genera *Klebsiella* and *Enterobacter*.
9. The lysine decarboxylase activity in *Salmonella* is used to differentiate this group from *Citrobacter freundii*. *Salmonella* Paratyphi A, however, gives an atypical negative reaction (yellow color of medium) in 24 hours when Decarboxylase Medium Base is used.[4]

References

1. Moeller. 1954. Acta. Pathol. Microbiol. Scand. *34*:102.
2. Moeller. 1954. Acta. Pathol. Microbiol. Scand. *34*:259.
3. Moeller. 1955. Acta. Pathol. Microbiol. Scand. *36*:158.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore, Md.
5. Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
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7. Mutters. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
8. Kiska and Gilligan. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
9. Falkow. 1958. Am. J. Clin. Pathol. *29*:598.
10. Ewing, Davis and Edwards. 1960. Publ. Health Lab. *18*:77.
11. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
12. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
13. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
14. Eaton, Rice and Baird (ed.) 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
15. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
16. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Decarboxylase Base Moeller

SMD SMWW USDA

Cat. No. 289020 Dehydrated – 500 g

BBL™ Moeller Decarboxylase Broth Base and Moeller Decarboxylase Broth with Amino Acids

SMD SMWW USDA

Cat. No.	211430	Dehydrated – 500 g*
	221731	Prepared Tubes, 5 mL – Pkg. of 10*
	221659	Prepared Tubes with Arginine, 5 mL – Pkg. of 10*
	221660	Prepared Tubes with Arginine, 5 mL – Ctn. of 100*
	221661	Prepared Tubes with Lysine, 5 mL – Pkg. of 10*
	221662	Prepared Tubes with Lysine, 5 mL – Ctn. of 100*
	221663	Prepared Tubes with Ornithine, 5 mL – Pkg. of 10*
	221664	Prepared Tubes with Ornithine, 5 mL – Ctn. of 100*

Difco™ Decarboxylase Medium Base

BAM CCAM COMPF ISO SMD SMWW

Cat. No. 287220 Dehydrated – 500 g

Difco™ Lysine Decarboxylase Broth

BAM CCAM COMPF ISO SMD SMWW

Cat. No. 211759 Dehydrated – 500 g

**Store at 2-8°C.*

D

Demi-Fraser Broth Base Fraser Broth Supplement

Intended Use

Demi-Fraser Broth Base is used with Fraser Broth Supplement in selectively and differentially enriching *Listeria* from foods.

Summary and Explanation

Fraser Broth Base and Fraser Broth Supplement are based on the Fraser Broth formulation of Fraser and Sperber.[1] The medium is used in the rapid detection of *Listeria* from food and environmental samples. Demi-Fraser Broth Base is a modification of Fraser Broth Base in which the nalidixic acid and acriflavine concentrations have been reduced to 10 mg/L and 12.5 mg/L respectively.[2]

Principles of the Procedure

Peptone, beef extract and yeast extract provide carbon and nitrogen sources and the cofactors required for good growth of *Listeria*. Sodium phosphate and potassium phosphate buffer the medium. Selectivity is provided by lithium chloride, nalidixic acid and acriflavine. The high sodium chloride concentration of the medium inhibits growth of enterococci.

All *Listeria* species hydrolyze esculin, as evidenced by a blackening of the medium. This blackening results from the formation of 6,7-dihydroxycoumarin, which reacts with ferric ions.[1] Ferric ions are added to the final medium as ferric ammonium citrate in Fraser Broth Supplement.

Formulae

Difco™ Demi-Fraser Broth Base

Approximate Formula* Per Liter		
Tryptose	10.0	g
Beef Extract	5.0	g
Yeast Extract	5.0	g
Sodium Chloride	20.0	g
Disodium Phosphate	9.6	g
Monopotassium Phosphate	1.35	g
Esculin	1.0	g
Nalidixic Acid	0.01	g
Acriflavine HCl	12.5	mg
Lithium Chloride	3.0	g

Difco™ Fraser Broth Supplement

Formula Per 10 mL Vial		
Ferric Ammonium Citrate	0.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 55 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
3. Aseptically add 10 mL of Fraser Broth Supplement. Mix well.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Demi-Fraser Broth Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in purified water. Solution is medium amber, clear to slightly opalescent, may have a fine precipitate.
Prepared Appearance:	Medium amber, very slightly to slightly opalescent, may have a fine precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Fraser Broth Supplement

Solution Appearance:	Dark brown solution.

Cultural Response

Difco™ Demi-Fraser Broth Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY/ APPEARANCE
Enterococcus faecalis	29212	10^3-2×10^3	Partial to complete inhibition
Escherichia coli	25922	10^3-2×10^3	Inhibition
Listeria monocytogenes	19114	10^2-10^3	Good/blackening of the medium
Listeria monocytogenes	19115	10^2-10^3	Good/blackening of the medium
Staphylococcus aureus	25923	10^3-2×10^3	Inhibition

Uninoculated Tube

Listeria monocytogenes ATCC™ 19114

Procedure[2]

1. Pre-enrich the sample in Demi-Fraser Broth. Incubate for 18-24 hours at 35 ± 2°C. Subculture onto Oxford Medium or PALCAM Medium.
2. Transfer 0.1 mL of the pre-enrichment culture into 10 mL of Fraser Broth and incubate for 48 hours at 37°C. Subculture onto Oxford Medium or PALCAM Medium after 18-24 hours and again after 42-48 hours of incubation.
3. Examine Oxford Medium or PALCAM Medium plates for the appearance of presumptive *Listeria* colonies.
4. Confirm the identity of all presumptive *Listeria* by biochemical and/or serological testing.

Expected Results

The presence of *Listeria* is presumptively indicated by the blackening of Demi-Fraser Broth after incubation for 24-48 hours at 35°C. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/ serological identification.

References

1. Fraser and Sperber. 1988. J. Food Prot. *51*:762.
2. L'association française de normalisation (AFNOR). 1993. Food microbiology- Detection of *Listeria monocytogenes*-Routine method, V 08-055. AFNOR, Paris, France.

Availability

Difco™ Demi-Fraser Broth Base

Cat. No.	265320	Dehydrated – 500 g
	265310	Dehydrated – 10 kg

Difco™ Fraser Broth Supplement

Cat. No.	211742	Tube – 6 x 10 mL*

**Store at 2-8°C.*

Dermatophyte Test Medium Base • Dermatophyte Test Medium, Modified with Chloramphenicol

Intended Use

Dermatophyte Test Medium (DTM) is a selective and differential medium used for the detection and presumptive identification of dermatophytes from clinical and veterinary specimens.[1] Because of the unavailability of one of the inhibitory agents, chlortetracycline, Dermatophyte Test Medium (DTM), Modified with Chloramphenicol is recommended as a substitute for the original DTM formation.

Summary and Explanation

Dermatophytes cause cutaneous fungal infections of the hair, skin and nails generally referred to as tinea or ringworm.[2-4]

User Quality Control

Identity Specifications

BBL™ Dermatophyte Test Medium Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.05% solution, soluble in purified water upon boiling. Solution is light to medium, yellow orange, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow orange, clear to slightly hazy.
Reaction of 4.05% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

BBL™ Dermatophyte Test Medium Base

Prepare the medium per label directions with added gentamicin sulfate-chloramphenicol solution. Inoculate with fresh cultures and incubate at 25 ± 2°C for 7 days.

ORGANISM	ATCC™	RECOVERY	REACTION
Aspergillus brasiliensis (niger)	16404	Partial to complete inhibition	–
Microsporum audouinii	9079	Fair to good	Alkaline (red)
Pseudomonas aeruginosa	10145	Partial to complete inhibition	–
Trichophyton mentagrophytes	9533	Fair to good	Alkaline (red)

Members of the genera *Trichophyton*, *Microsporum* and *Epidermophyton* are the most common etiologic agents of these infections.

Taplin et al. developed DTM as a screening medium for the selective isolation and detection of dermatophytes from clinical specimens.[5] A combination of three antimicrobial agents (cycloheximide, chlortetracycline and gentamicin) inhibited bacteria and saprophytic yeasts and molds. Lack of availability of chlortetracycline in late 1992 resulted in the substitution of chloramphenicol for chlortetracycline.

Dermatophytes are presumptively identified based on gross morphology and the production of alkaline metabolites, which raise the pH and cause the phenol red indicator to change the color of the medium from yellow to pink to red.[3-5] Taplin et al. reported the medium (with chlortetracycline) to be 97 to 100% accurate for identifying dermatophytes.[5]

Principles of the Procedure

The soy peptone provides nitrogenous and carbonaceous substances essential for microbial growth. Dextrose is a source of energy for metabolism. The pH indicator, phenol red, is used to detect acid production.

Cycloheximide inhibits most saprophytic molds. The additives, gentamicin and chloramphenicol, aid in the selectivity of the medium. Gentamicin inhibits gram-negative bacteria including *Pseudomonas* species. Chloramphenicol is a broad-spectrum antibiotic that inhibits a wide range of gram-positive and gram-negative bacteria.

Formula

BBL™ Dermatophyte Test Medium Base

Approximate Formula* Per Liter		
Papaic Digest of Soybean Meal	10.0	g
Dextrose	10.0	g
Phenol Red	0.2	g
Cycloheximide	0.5	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 40.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and add gentamicin sulfate and chloramphenicol (0.1 g of each per L).
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the specimen as soon as possible after it is received in the laboratory. Implant cutaneous specimens by gently pressing the samples into the agar surface.

For isolation of fungi from potentially contaminated specimens, a nonselective medium should be inoculated along with the selective medium. Incubate plates at 22-25°C in an inverted position (agar side up) with increased humidity and tubes with caps loosened to allow air to circulate.

Expected Results

Dermatophytes produce typical morphology and a pink to red color in the medium around the colony within 10-14 days of incubation. Disregard color changes after the fourteenth day of incubation because they may be caused by contaminating fungi.[5]

Certain strains of *Candida albicans* are capable of converting the indicator to red, but the yeast can be recognized by their white bacteria-like colonial appearance. Certain nondermatophyte fungi rarely can produce alkaline products (false positives).

Inoculation onto conventional media is recommended for definitive identification of isolates presumptively identified as dermatophytes.

Limitation of the Procedure

The complete classification of dermatophytes depends on microscopic observations of direct and slide culture preparations along with biochemical and serological tests.[6]

References

1. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
2. Forbes, Sahm and Weissfeld. 1994. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
3. Kane and Summerbell. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
5. Taplin, Zaias, Rebell and Blank. 1969. Arch. Dermatol. 99:203-209.
6. Larone. 1995. Medically important fungi: a guide to identification, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Dermatophyte Test Medium Base

Cat. No. 212330 Dehydrated – 500 g

BBL™ Dermatophyte Test Medium, Modified with Chloramphenicol

BS12 MCM9

United States and Canada

Cat. No. 299636 Prepared Plates – Pkg. of 10*
299701 Prepared Slants – Pkg. of 10*

Europe

Cat. No. 254429 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Desoxycholate Agar

Intended Use

Desoxycholate Agar is a slightly selective and differential plating medium used for isolating and differentiating gram-negative enteric bacilli.

Summary and Explanation

Desoxycholate Agar as formulated by Leifson[1] demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora. The medium was an improvement over other media of the time because the chemicals, citrates and sodium desoxycholate, in specified amounts, worked well as inhibitors. This medium has been used to screen for *Salmonella* spp. and *Shigella* spp. from clinical specimens.[2]

Principles of the Procedure

Peptone provides nitrogen and carbon for general growth requirements. Lactose is the fermentable carbohydrate. Sodium chloride and dipotassium phosphate maintain the osmotic balance of the medium. Sodium desoxycholate, ferric citrate and sodium citrate inhibit growth of gram-positive bacteria. Neutral red is a pH indicator. Agar is the solidifying agent.

Differentiation of enteric bacilli is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of neutral red, form red colonies. Bacteria that do not ferment lactose form colorless colonies. The majority of normal intestinal bacteria ferment lactose (red colonies), while *Salmonella* and *Shigella* species do not ferment lactose (colorless colonies).

User Quality Control

Identity Specifications

Difco™ Desoxycholate Agar

Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is reddish-orange, slightly opalescent.
Prepared Appearance:	Orange, slightly opalescent.
Reaction of 4.5% Solution at 25°C:	pH 7.3 ± 0.2

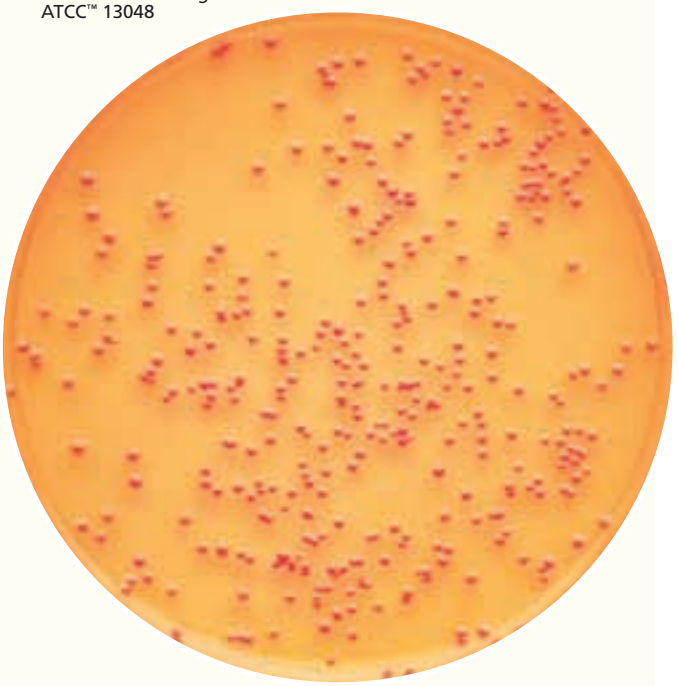
Cultural Response

Difco™ Desoxycholate Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^3-2×10^3	Marked inhibition	–
Escherichia coli	25922	30-300	Good	Pink w/bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless

Enterobacter aerogenes
ATCC™ 13048



Formula

Difco™ Desoxycholate Agar

Approximate Formula* Per Liter		
Peptone	10.0	g
Lactose	10.0	g
Sodium Desoxycholate	1.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.0	g
Ferric Ammonium Citrate	1.0	g
Sodium Citrate	1.0	g
Agar	15.0	g
Neutral Red	0.03	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the isolation of enteric bacilli, refer to appropriate procedures outlined in the references.[2-4]

Expected Results

Refer to appropriate references and procedures for results.[2-4]

References

1. Leifson. 1935. J. Pathol. Bacteriol. *40*:581.
2. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
4. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Desoxycholate Agar

COMPF SMWW

Cat. No. 227310 Dehydrated – 500 g

Europe

Cat. No. 254010 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251550 Prepared Plates – Pkg. of 20*
251824 Prepared Plates – Ctn. of 200*
251507 Prepared **RODAC**™ Plates – Pkg. of 30*

**Store at 2-8°C.*

D

Desoxycholate Citrate Agar

Intended Use

Desoxycholate Citrate Agar is a moderately selective and differential plating medium used for isolating enteric bacilli, particularly *Salmonella* and many *Shigella* species.

Summary and Explanation

Desoxycholate Citrate Agar is a modification of Desoxycholate Agar formulated by Leifson.[1] His original medium demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using citrates and sodium desoxycholate in specified amounts as inhibitors to gram-positive bacteria.

Leifson modified his original medium by increasing the concentration of sodium citrate and sodium desoxycholate and found Desoxycholate Citrate Agar reliable for isolating many *Salmonella* and *Shigella* species.[1]

Desoxycholate Citrate Agar effectively isolates intestinal pathogens (*Salmonella* and *Shigella* species) by inhibiting coliforms and many *Proteus* species.

Principles of the Procedure

Infusion from meat is a source of carbon and nitrogen. This ingredient is used because the inhibition of coliforms produced is greater than when an extract or simple peptone is used.[1] Peptone provides carbon, nitrogen, vitamins and minerals. Lactose is the fermentable carbohydrate. Sodium citrate and sodium desoxycholate inhibit gram-positive bacteria, coliforms and *Proteus* species. Ferric ammonium citrate aids in the detection of H_2S-producing bacteria. Neutral red is a pH indicator. Agar is the solidifying agent.

In the presence of neutral red, bacteria that ferment lactose produce acid and form red colonies. Bacteria that do not ferment lactose form colorless colonies. If the bacteria produce H_2S, the colonies will have black centers. The majority of normal intestinal bacteria ferment lactose and do not produce H_2S (red colonies without black centers). *Salmonella* and *Shigella* spp. do not ferment lactose but *Salmonella* may produce H_2S (colorless colonies with or without black centers). Lactose-fermenting colonies may have a zone of precipitation around them caused by the precipitation of desoxycholate in the presence of acid.

Formula

Difco™ Desoxycholate Citrate Agar

Approximate Formula* Per Liter		
Meat, Infusion from 330 g	9.5	g
Proteose Peptone No. 3	10.0	g
Lactose	10.0	g
Sodium Citrate	20.0	g
Ferric Ammonium Citrate	2.0	g
Sodium Desoxycholate	5.0	g
Agar	13.5	g
Neutral Red	0.02	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Desoxycholate Citrate Agar

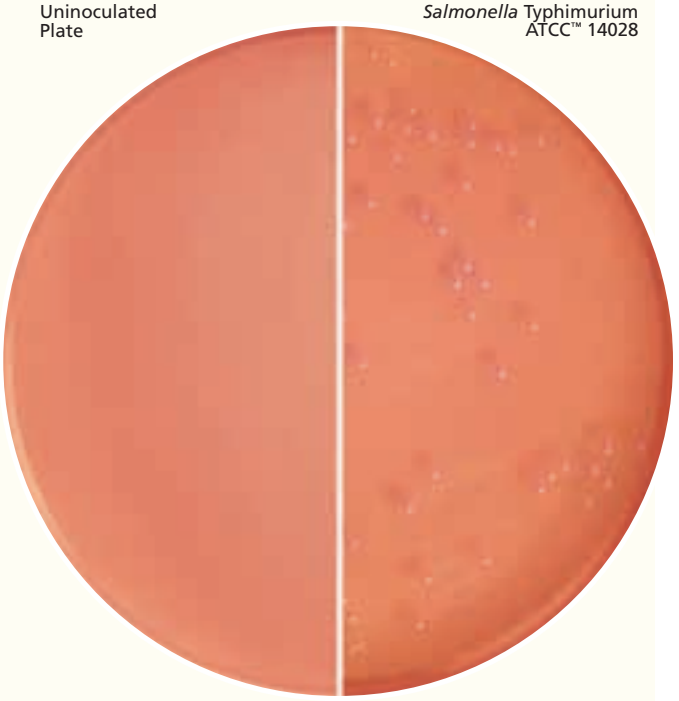
Dehydrated Appearance:	Pinkish-beige, free-flowing, homogeneous.
Solution:	7.0% solution, soluble in purified water upon boiling. Solution is orange-red, very slightly to slightly opalescent.
Prepared Appearance:	Orange-red, slightly opalescent.
Reaction of 7.0% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Difco™ Desoxycholate Citrate Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	H_2S
Enterococcus faecalis	29212	10^3-2×10^3	Marked to complete inhibition	–	–
Escherichia coli	25922	10^2-10^3	Partial to complete inhibition	Pink with bile precipitate	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Fair to good	Colorless	+
Shigella flexneri	12022	10^2-10^3	Fair	Colorless	–



Directions for Preparation from Dehydrated Product

1. Suspend 70 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Inoculate specimen directly onto surface of medium.
2. Incubate plates at 35 ± 2°C for 18-24 hours. Plates can be incubated for an additional 24 hours if no lactose fermenters are observed.

Expected Results

Lactose nonfermenters produce transparent, colorless to light pink or tan colored colonies with or without black centers. Lactose fermenters produce a red colony with or without a bile precipitate.

Limitations of the Procedure

1. Coliform strains may be encountered that will grow on this medium, making it difficult to detect pathogens.
2. Heavy inocula should be distributed over the entire surface of the medium to prevent complete masking of pathogens by coliform organisms.

Reference

1. Leifson. 1935. J. Pathol. Bacteriol. *40*:581.

Availability

Difco™ Desoxycholate Citrate Agar

EP

Cat. No. 227410 Dehydrated – 500 g

Desoxycholate Lactose Agar

Intended Use

Desoxycholate Lactose Agar is a slightly selective and differential plating medium used for isolating and differentiating gram-negative enteric bacilli and for enumerating coliforms from water, wastewater, milk and dairy products.

Summary and Explanation

Desoxycholate Lactose Agar is a modification of Desoxycholate Agar formulated by Leifson.[1] His original medium demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using citrates and sodium desoxycholate in specified amounts as inhibitors to gram-positive bacteria.

Standard methods manuals for dairy[2] and water[3] specified a modification of Desoxycholate Agar to contain less sodium desoxycholate and, accordingly, be less inhibitory to gram-positive bacteria. This formulation, known as Desoxycholate Lactose Agar, was used in pour plate procedures for isolation and enumeration of coliforms in milk, water and other specimens. The medium is no longer included in recent editions of these manuals.

Principles of the Procedure

Peptone provides nitrogen and carbon for general growth requirements. Lactose is a fermentable carbohydrate. Sodium chloride maintains the osmotic balance of the medium. Sodium desoxycholate and sodium citrate inhibit growth of gram-positive bacteria. Neutral red is a pH indicator. Agar is the solidifying agent.

Differentiation of enteric bacilli is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of neutral red, form red colonies. Bacteria that do not ferment lactose form colorless colonies. The majority of normal intestinal bacteria ferment lactose (red colonies) while *Salmonella* and *Shigella* species do not ferment lactose (colorless colonies).

Formula

Difco™ Desoxycholate Lactose Agar

Approximate Formula* Per Liter		
Proteose Peptone	10.0	g
Lactose	10.0	g
Sodium Desoxycholate	0.5	g
Sodium Chloride	5.0	g
Sodium Citrate	2.0	g
Agar	15.0	g
Neutral Red	0.03	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 42.5 g of the powder in 1 L of purified water. Mix thoroughly.

User Quality Control

Identity Specifications

Difco™ Desoxycholate Lactose Agar

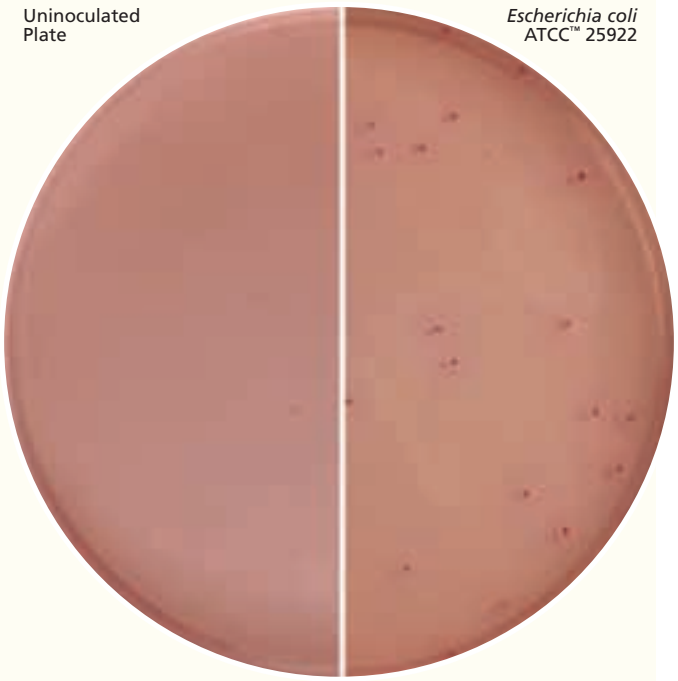
Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous.
Solution:	4.25% solution, soluble in purifed water upon boiling. Solution is pinkish-red, very slightly to slightly opalescent.
Prepared Appearance:	Pinkish-red, very slightly to slightly opalescent.
Reaction of 4.25% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Difco™ Desoxycholate Lactose Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Bacillus subtilis	6633	~10^3	Inhibition	–
Enterobacter aerogenes	13048	30-300	Good	Pink, may have slight bile precipitate
Enterococcus faecalis	29212	~10^3	Inhibition	–
Escherichia coli	25922	30-300	Good	Pink w/bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless



2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
See appropriate references for specific procedures.[2,3]

Expected Results
Refer to appropriate references and procedures for results.[2,3]

References
1. Leifson. 1935. J. Pathol. Bacteriol. *40*:581.
2. American Public Health Association. 1960. Standard methods for the examination of dairy products, 11th ed. American Public Health Association, New York, N.Y.
3. American Public Health Association. 1960. Standard methods for the examination of water and wastewater, 11th ed. American Public Health Association, New York, N.Y.

Availability
Difco™ Desoxycholate Lactose Agar
Cat. No. 242010 Dehydrated – 500 g

Dextrose Agar • Dextrose Broth

Intended Use
Dextrose Agar is used for cultivating a wide variety of microorganisms with or without added blood.

Dextrose Broth is used for cultivating fastidious microorganisms and for detecting gas from enteric bacilli.

Summary and Explanation
In 1932, Norton[1] recommended a basal medium containing 0.5-1% dextrose with approximately 5% defibrinated blood for the isolation of many fastidious bacteria, including *Haemophilus* and *Neisseria*. Dextrose is an energy source used by many organisms. The high concentration of this ingredient makes Dextrose Agar a suitable medium for the production of early, abundant organism growth and shortening the lag periods of older cultures. Because of the increased dextrose content, Dextrose Agar is not suitable for observation of hemolysis when supplemented with 5% sheep, rabbit or horse blood.

Dextrose Broth is a highly nutritious broth suitable for the isolation of fastidious organisms and specimens containing a low inoculum. The addition of 0.1-0.2% agar to Dextrose Broth facilitates anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment.[2] The low agar concentration provides suitable conditions for both aerobic growth in the clear upper zone and for microaerophilic and anaerobic growth in the lower, flocculent agar zones.

User Quality Control

Identity Specifications

Difco™ Dextrose Agar

Dehydrated Appearance:	Medium beige, homogeneous, free-flowing.
Solution:	4.3% solution, soluble in purified water upon boiling. Solution is medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Plain – Light to medium amber, slightly opalescent without significant precipitate.
	With blood – Cherry-red, opaque.
Reaction of 4.3% Solution at 25°C:	pH 7.3 ± 0.2

Difco™ Dextrose Broth

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
Solution:	2.3% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 2.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Dextrose Agar

Prepare the medium per label directions without (plain) and with sterile 5% defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Escherichia coli	25922	10^2-10^3	Good	Good
Neisseria meningitidis	13090	10^2-10^3	Poor	Good
Staphylococcus aureus	25923	10^2-10^3	Good	Good
Streptococcus pneumoniae	6305	10^2-10^3	Fair	Good
Streptococcus pyogenes	19615	10^2-10^3	Good	Good

Difco™ Dextrose Broth

Prepare the medium per label directions with one set of tubes containing fermentation vials and a second set of tubes (without fermentation vials) containing medium supplemented with 0.1% agar. Inoculate and incubate at 35 ± 2°C. Read growth and gas production at 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS	RECOVERY W/0.1% AGAR
Escherichia coli	25922	10^2-10^3	Good	+	Good
Neisseria meningitidis	13090	10^2-10^3	Good	–	Good
Staphylococcus aureus	25923	10^2-10^3	Good	–	Good
Streptococcus pneumoniae	6305	10^2-10^3	Good	–	Good
Streptococcus pyogenes	19615	10^2-10^3	Good	–	Good

Dextrose Agar and Dextrose Broth are specified in the *Compendium of Methods for the Microbiological Examination of Foods*.[3]

Principles of the Procedure

Beef extract and peptones provide nitrogen, amino acids and vitamins. Dextrose is a carbon source, and the increased concentration is a distinguishing characteristic of this medium from other formulations used as blood agar bases. Agar is the solidifying agent.

Supplementation with 5% blood provides additional growth factors for fastidious microorganisms.

Formulae

Difco™ Dextrose Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	2.0	g
Pancreatic Digest of Gelatin	3.0	g
Beef Extract	3.0	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

Difco™ Dextrose Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	2.0	g
Pancreatic Digest of Gelatin	3.0	g
Beef Extract	3.0	g
Dextrose	5.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Dextrose Agar

1. Suspend 43 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well and dispense as desired.

Difco™ Dextrose Broth

1. Dissolve 23 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

OPTIONAL: To prepare medium with agar, add 1-2 g of agar per liter of medium.

Procedure

For a complete discussion on microorganism isolation and identification, refer to appropriate references.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Norton. 1932. J. Lab. Clin. Med. *17*:558.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Dextrose Agar

COMPF

Cat. No. 267100 Dehydrated – 500 g

Difco™ Dextrose Broth

COMPF

Cat. No. 263100 Dehydrated – 500 g

Dextrose Starch Agar

Intended Use

Dextrose Starch Agar is used for cultivating pure cultures of *Neisseria gonorrhoeae* and other fastidious microorganisms.

Summary and Explanation

Dextrose Starch Agar is recommended as a complete solid medium for the propagation of pure cultures of *Neisseria gonorrhoeae*. This highly nutritious medium without additives will also support excellent growth of *N. meningitidis*, *Streptococcus pneumoniae* and *S. pyogenes*. Dextrose Starch Agar, in half concentration, is recommended as a stock culture agar for the maintenance of *N. gonorrhoeae*, *N. meningitidis* and other organisms not capable of hydrolyzing starch. This medium cannot be used to maintain stock cultures of organisms capable of splitting starch; acid production from starch will create an unsatisfactory environment.

Dextrose Starch Agar was used by Wilkins, Lewis and Barbiers[1] in an agar dilution procedure to test the activity of antibiotics against *Neisseria* species.

Principles of the Procedure

Peptone and gelatin provide the nitrogen, vitamins and amino acids in Dextrose Starch Agar. Soluble starch improves growth response. Dextrose is a carbon source. Sodium chloride

User Quality Control

Identity Specifications

Difco™ Dextrose Starch Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is light amber, opalescent with a precipitate.
Prepared Appearance:	Light amber, opalescent with a precipitate.
Reaction of 6.5% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Dextrose Starch Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria gonorrhoeae	43070	10^2-10^3	Good
Neisseria meningitidis	13090	10^2-10^3	Good
Pasteurella multocida	19427	10^2-10^3	Good
Streptococcus pneumoniae	6303	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

maintains the osmotic balance of the medium, and disodium phosphate is a buffering agent. Agar is the solidifying agent.

Formula

Difco™ Dextrose Starch Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	15.0	g
Dextrose	2.0	g
Soluble Starch	10.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	3.0	g
Gelatin	20.0	g
Agar	10.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 65 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion of the isolation and identification of *N. gonorrhoeae* and other fastidious pathogens, refer to the procedures described in *Clinical Microbiology Procedures Handbook*[2] and *Manual of Clinical Microbiology*.[3]

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

This medium is not recommended for isolation of gonococci from mixed cultures.

References

1. Wilkins, Lewis and Barbiers. 1956. Antibiot. Chemother. 6:149.
2. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Dextrose Starch Agar

Cat. No. 266200 Dehydrated – 500 g

Dextrose Tryptone Agar

Intended Use

Dextrose Tryptone Agar is used for cultivating thermophilic "flat-sour" microorganisms associated with food spoilage.

Summary and Explanation

In the 1930s, the National Canners Association specified the use of Dextrose Tryptone Agar for isolating "flat sour" organisms from food products.[1] "Flat sour" spoilage of canned foods is caused by *Bacillus coagulans* (*Bacillus thermoacidurans*). Bacterial growth results in a 0.3-0.5 drop in pH, while the ends of the can remain flat. *B. coagulans* is a soil microorganism that can be found in canned tomato products and dairy products. Conditions favorable for multiplication of the bacterium can result in spoilage of the food product.[2]

Dextrose Tryptone Agar can also be used to isolate other food spoilage bacteria: mesophilic aerobic spore formers in the genera *Bacillus* and *Sporolactobacillus* and thermophilic flat sour spore formers such as *B. stearothermophilus*.[2]

Principles of the Procedure

Dextrose Tryptone Agar contains peptones to provide carbon and nitrogen sources for general growth requirements. Dextrose is the carbohydrate source. Bromcresol purple is the pH indicator. Agar is the solidifying agent.

Formula

Difco™ Dextrose Tryptone Agar

Approximate Formula* Per Liter		
Tryptone	8.0	g
Peptone	2.0	g
Dextrose	5.0	g
Agar	15.0	g
Bromcresol Purple	0.04	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Dextrose Tryptone Agar

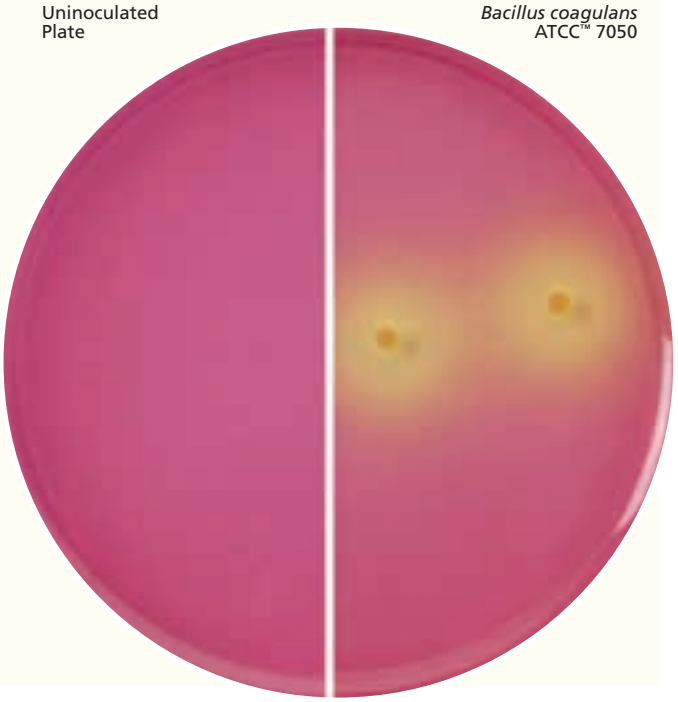
Dehydrated Appearance:	Light, greenish-beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in purified water upon boiling. Solution is purple, slightly opalescent.
Prepared Appearance:	Purple, slightly opalescent without significant precipitate.
Reaction of 3.0% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Dextrose Tryptone Agar

Prepare the medium per label directions. Inoculate plates by the pour plate method and incubate at 55 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	DEXTROSE FERMENTATION
Bacillus coagulans	7050	10^2-10^3	Good	+ (yellow)
Bacillus stearothermophilus	7953	10^2-10^3	Good	+ (yellow)



Directions for Preparation from Dehydrated Product

1. Suspend 30 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

A change in the color of the medium from purple to yellow indicates dextrose fermentation.

References

1. National Canners Association. 1933. Bacterial standards for sugar.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Dextrose Tryptone Agar

COMPF

Cat. No. 280100 Dehydrated – 500 g

Differential Reinforced Clostridial Agar

Intended Use

Differential Reinforced Clostridial Agar is used for enumerating and cultivating sulfite-reducing clostridia.

Summary and Explanation

Differential Reinforced Clostridial Medium (broth) was developed by Gibbs and Freame in 1965.[1] The medium could be used to enumerate clostridia in foods using the Most Probable Number (MPN) method. Differential Reinforced Clostridial Agar (DRCA) is based on Differential Reinforced Clostridial Medium, but with the addition of agar.

The assay is performed using unheated and heat-shocked tubes of DRCA containing replicate dilutions of the test sample. Blackening of the medium is presumptive evidence for the presence of sulfite-reducing clostridia. In this method, heat-shocked tubes showing blackening are confirmatory for clostridia. Non-heat-shocked tubes showing blackening must be heat shocked to kill off vegetative cells and subcultured into DRCA to confirm the presence of sulfite-reducing clostridia.

Principles of the Procedure

Peptones, beef extract, yeast extract, starch and L-cysteine provide nutrients and co-factors required for good growth of clostridia. Dextrose is included in the medium as an energy source. Partial selectivity of the medium is achieved through the addition of sodium acetate. Agar is the solidifying agent. Anaerobiosis in the medium is detected by the redox indicator resazurin. The addition of ferric ammonium citrate to the medium is used to detect sulfite reduction. Blackening of the medium is due to the formation of iron sulfide.

User Quality Control

Identity Specifications

Difco™ Differential Reinforced Clostridial Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.25% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent while hot; upon cooling, solution becomes light red.
Prepared Appearance:	Light pink, clear to slightly opalescent without significant precipitate.
Reaction of 4.25% Solution at 25°C:	pH 7.1 ± 0.2

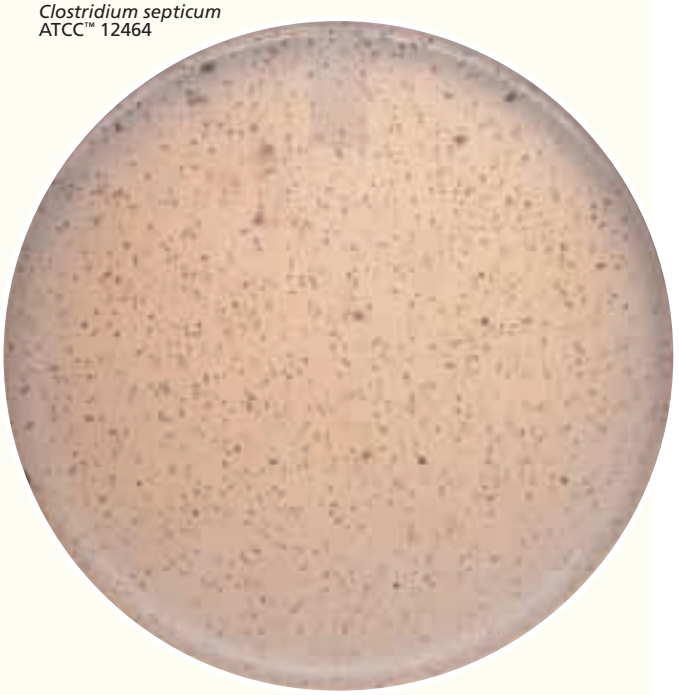
Cultural Response

Difco™ Differential Reinforced Clostridial Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C in an anaerobic atmosphere for 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	BLACK COLONIES
Clostridium bifermentans	638	10^2-10^3	Good	+
Clostridium perfringens	12924	10^2-10^3	Good	+
Clostridium septicum	12464	10^2-10^3	Good	+

Clostridium septicum
ATCC™ 12464



Formula

Difco™ Differential Reinforced Clostridial Agar

Approximate Formula* Per Liter

Tryptone	5.0	g
Peptone	5.0	g
Beef Extract, Desiccated	8.0	g
Yeast Extract	1.0	g
L-Cysteine HCl	0.5	g
Starch	1.0	g
Dextrose	1.0	g
Sodium Acetate	5.0	g
Sodium Bisulfite	0.5	g
Ferric Ammonium Citrate	0.5	g
Resazurin	2.0	mg
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 42.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Prepare serial 10-fold dilutions of the sample in 1/4 strength Ringer's solution or 0.1% peptone water.
2. Depending on the amount of the initial sample, transfer 1 mL or 0.1 mL of the appropriate dilution, prepared in step 1, to the bottom of a molten (45-50°C) DRCA tube. Prepare a duplicate tube using the same procedure.
3. Tighten the caps on the tubes.
4. Heat one of the duplicate DRCA tubes prepared in step 2 to 80 ± 1°C for 10 minutes to kill vegetative cells.
5. Incubate both tubes, heat-shocked and non-heat-shocked, at 35 ± 1°C for 5 days; examine for sulfite reduction.

Non-heat-shocked cultures showing blackening must be heat shocked and subcultured to DRCA for confirmation.

Alternative Procedures

Inoculate samples onto the surface of agar plates using the streak plate or spread plate technique. Samples may be inoculated into DRCA using the pour plate technique. Medium in agar deeps may be inoculated using the stab technique. DRCA may be used to overlay the membrane filter in the membrane filter technique. Incubate plates and tubes at 35 ± 1°C for 24-48 hours under anaerobic conditions. Agar deeps may be incubated under aerobic conditions when following the Prickett tube method.[2]

Expected Results

The presence of clostridia is presumptively indicated by blackening in the medium. Heat-shocked tubes showing blackening should be considered confirmatory for the presence of sulfite-reducing clostridia.

References

1. Gibbs and Freame. 1965. J. Appl. Microbiol. *28*:95.
2. Miller, Gerrett and Prickett. 1939. Food Res. *4*:447.

Availability

Difco™ Differential Reinforced Clostridial Agar

Cat. No. 264120 Dehydrated – 500 g

Dubos Media
Dubos Broth Base • Dubos Medium Albumin Dubos Oleic Agar Base • Dubos Oleic Albumin Complex • Dubos Broth, Enriched

Intended Use

Dubos Broth Base is used with Dubos Medium Albumin for rapidly cultivating pure cultures of *Mycobacterium tuberculosis*.

Dubos Oleic Agar Base is used with Dubos Oleic Albumin Complex and penicillin for isolating and determining the susceptibility of *M. tuberculosis*.

Dubos Broth, Enriched is a prepared medium used for the cultivation of pure cultures of *M. tuberculosis*.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] During the mid 1980s, the number of tuberculosis (TB) cases in the U.S. began increasing. Prior to this time, the number of cases in the U.S. had been decreasing, reaching a low in 1984.[2] Non-tuberculous mycobacterial infections have also increased since the mid 1980s.[3]

Dubos Broth is prepared according to the Dubos, Fenner and Pierce[4] modification of the medium originally described by Dubos and Davis[5] and Dubos and Middlebrook.[6]

Dubos and Middlebrook[6] described Dubos Oleic Medium Albumin as suitable for primary isolation and cultivation of the tubercle bacillus and for studying colony morphology. In comparative studies, Dubos Oleic Albumin Agar Medium was superior to other media studied for primary isolation.[7,8]

There are two types of solid culture media for the primary isolation of mycobacteria, those that have coagulated egg as a base and those that have agar. Lowenstein formulations are examples of media that contain egg; Middlebrook and Dubos formulations contain agar.

Agar based media are not liquefied by contaminating proteolytic organisms but overgrowth may occur. These media are recommended for specimens from nonsterile sites.[9] The medium is clear so colonies of mycobacteria can be viewed through a stereo microscope even if contaminating organisms are present. Colonies can be observed in 10-12 days.

Drugs may be added to Dubos media in exact concentrations because the medium is solidified with agar rather than by inspissation. Also, there is less drug inactivation when egg ingredients are not present.

Mycobacteria grow more rapidly in broth media. Primary culture of all specimens in broth media is recommended.[10] Polysorbate 80 in the medium acts as a surfactant, dispersing the bacilli, which increases growth.

Dubos Broth, Enriched is a modified medium based on the formulation of Dubos et al.[4] This formulation differs from the original in that it has a strong buffering system and an acid pH.[11] The particular value of Dubos Broth, Enriched is that it provides dispersed growth, free of excessive clumps, which can be used to prepare a relatively uniform suspension of mycobacteria for use in bacterial studies. It is also used as a subculture and enrichment medium for the rapid cultivation of *M. tuberculosis* and other mycobacterial species from treated clinical specimens and from direct inoculation of specimens that may yield pure cultures; e.g., cerebrospinal fluid.[12]

Principles of the Procedure

Peptone and asparagine are sources of nitrogen. Disodium phosphate and monopotassium phosphate are sources of phosphates and, along with calcium chloride, help maintain the pH of the medium. Magnesium sulfate, ferric ammonium sulfate, zinc sulfate and copper sulfate are sources of trace metals and sulfates. Polysorbate 80, an oleic acid ester, supplies essential fatty acids for the replication of mycobacteria. Bovine albumin acts as a protective agent by binding free fatty acids that may be toxic to mycobacteria. The albumin is heat-treated to inactivate lipase, which may release fatty acids from the polysorbate 80. Phosphate buffers maintain the pH of the medium. Agar is the solidifying agent.

Formulae

Difco™ Dubos Broth Base

Approximate Formula* Per Liter

Pancreatic Digest of Casein	0.5	g
Asparagine	2.0	g
Polysorbate 80	0.2	g
Monopotassium Phosphate	1.0	g
Disodium Phosphate (anhydrous)	2.5	g
Ferric Ammonium Citrate	50.0	mg
Magnesium Sulfate	10.0	mg
Calcium Chloride	0.5	mg
Zinc Sulfate	0.1	mg
Copper Sulfate	0.1	mg

Difco™ Dubos Medium Albumin

A 5% solution of albumin fraction V from bovine plasma and 7.5% dextrose in normal saline (0.85%).

User Quality Control

Identity Specifications

Difco™ Dubos Broth Base

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	0.65 g/90 mL solution, soluble in purified water upon boiling. Solution is very light to light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light to light amber, clear, may have a slight precipitate.
Reaction of 0.65 g/90 mL Solution at 25°C:	pH 6.6 ± 0.2

Difco™ Dubos Oleic Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent to opalescent with fine precipitate.
Prepared Appearance:	Light amber, slightly opalescent to opalescent with fine precipitate.
Reaction of 2.0% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Difco™ Dubos Broth Base

Prepare the medium per label directions with added Dubos Medium Albumin (10 mL of albumin to 90 mL base). Inoculate and incubate at 35 ± 2°C with 5-10% CO_2 for up to 3 weeks.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Mycobacterium fortuitum	6841	~10^3	Good
Mycobacterium intracellulare	13950	~10^3	Good
Mycobacterium kansasii	12478	~10^3	Good
Mycobacterium scrofulaceum	19981	~10^3	Good
Mycobacterium tuberculosis H37Ra	25177	~10^3	Good

Difco™ Dubos Oleic Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C with 5-10% CO_2 for up to 3 weeks.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	~10^3	Partial inhibition
Mycobacterium fortuitum	6841	~300	Good
Mycobacterium intracellulare	13950	~300	Good
Mycobacterium kansasii	12478	~300	Good
Mycobacterium scrofulaceum	19981	~300	Good
Mycobacterium tuberculosis H37Ra	25177	~300	Good

Difco™ Dubos Oleic Agar Base

Approximate Formula* Per Liter

Pancreatic Digest of Casein	0.5	g
Asparagine	1.0	g
Monopotassium Phosphate	1.0	g
Disodium Phosphate (anhydrous)	2.5	g
Agar	15.0	g
Ferric Ammonium Citrate	50.0	mg
Magnesium Sulfate	10.0	mg
Calcium Chloride	0.5	mg
Zinc Sulfate	0.1	mg
Copper Sulfate	0.1	mg

Difco™ Dubos Oleic Albumin Complex

A 0.05% solution of alkalinized oleic acid in a 5% solution of albumin fraction V in normal saline (0.85%).

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions[13,14]

1. Biosafety Level 2 practices, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet.
2. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activites in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Directions for Preparation from Dehydrated Product

Difco™ Dubos Broth Base

1. Suspend 1.3 g of the powder in 180 mL of purified water (or 170 mL of purified water and 10 mL glycerol). Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to below 50°C.
4. Aseptically add 20 mL Dubos Medium Albumin and mix thoroughly. Incubate medium for 24 hours to test for microbial load.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Dubos Oleic Agar Base

1. Suspend 4 g of the powder in 180 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to below 50-55°C.
4. Aseptically add 20 mL Dubos Oleic Albumin Complex and 5,000-10,000 units of penicillin (25-50 units per mL of medium). Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The test procedures are recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[13] N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle, but effective digesting and decontaminating agent. These reagents are provided in the **BBL™ MycoPrep™** Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate text.[3,9,12,13,15]

Specimens that are less likely to be contaminated with other microorganisms (cerebrospinal fluid, pleural fluid, tissue biopsy, etc.) may be inoculated directly into the medium. Consult appropriate texts for recommended procedures.[3,9,12,13,15]

Incubate the tubes at 35 ± 2°C in a CO_2-enriched atmosphere. Keep the tube caps loosened for at least one week to permit circulation of CO_2, but tighten the caps thereafter to prevent dehydration. Loosen briefly once a week to replenish CO_2. Six to eight weeks of incubation may be necessary for evidence of growth of many mycobacteria.

Expected Results

Growth of mycobacterial colonies on the agar medium or in broth media, as indicated by turbidity compared to an uninoculated control.

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

1. Musser. 1995. Clin. Microbiol. Rev. *8*:496.
2. Klietmann. 1995. Clin. Microbiol. Newsl. *17*:65.
3. Metchock, Nolte and Wallace. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Dubos, Fenner and Pierce. 1950. Am. Rev. Tuberc. *61*:66.
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7. Roberts, Wallace and Erlich. 1950. Am. Rev. Tuberc. *61*:563.
8. Byham. 1950. Am. J. Clin. Pathol. *20*:678.
9. Isenberg (ed.). 1994. Clinical microbiology procedures handbook, suppl. 1. American Society for Microbiology, Washington, D.C.
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12. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. Ed. Weissfeld, American Society for Microbiology, Washington, D.C.
13. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS, Centers for Disease Control, Atlanta, Ga.
14. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
15. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.

Availability

Difco™ Dubos Broth Base

Cat. No. 238510 Dehydrated – 500 g

Difco™ Dubos Medium Albumin

AOAC

Cat. No. 230910 Tube, 20 mL – Pkg. of 12*

Difco™ Dubos Oleic Agar Base

Cat. No. 237310 Dehydrated – 500 g

Difco™ Dubos Oleic Albumin Complex

Cat. No. 237510 Tube, 20 mL – Pkg. of 12*

BBL™ Dubos Broth, Enriched

Cat. No. 295697 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*

E

m E Agar • Esculin Iron Agar

Intended Use

m E Agar is used with nalidixic acid and triphenyltetrazolium chloride in isolating and differentiating enterococci from water by membrane filtration and in an *in situ* esculin test on Esculin Iron Agar.

Esculin Iron Agar (EIA substrate) is used for enumerating enterococci from water by membrane filtration based on esculin hydrolysis.

Summary and Explanation

Enterococcus species are a subgroup of fecal streptococci that includes *E. faecalis*, *E. faecium*, *E. gallinarum* and *E. avium*.[1] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10°C and 45°C.[1] The enterococci portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1]

Slanetz and Bartley[2] first reported quantitating enterococci by the membrane filter method in 1957. A wide range of levels of enterococci in water can be enumerated and detected because small or large volumes of water can be analyzed by the membrane filter technique.[3] In 1961, Kenner et al.[4] described the KF method for detecting and quantitating fecal streptococci. In 1966, Isenberg et al.[5] reported a plating procedure with differentiation based on esculin hydrolysis. Levin, Fischer and Cabelli[6] compared the KF method with Isenberg's plating method, and found the latter method resulted in better recovery of fecal streptococci. They developed m E Agar as a primary isolation medium for enterococci, and Esculin Iron Agar as an *in situ* substrate test medium for identifying organisms capable of hydrolyzing esculin.[6]

Two research projects by the U.S. Environmental Protection Agency (USEPA) evaluated the relationships between swimming-associated illness and the ambient densities of indicator bacteria.[7,8] The studies demonstrated that enterococci have a better correlation with swimming-associated illness for both marine and fresh waters than fecal coliforms. *Escherichia coli* has a correlation in fresh water equal to enterococci but does not correlate as well in marine waters.[7,8] This suggests that enterococci may be better indicator organisms for some recreational waters.[7,8]

m E Agar and Esculin Iron Agar are prepared according to the formulas specified in standard methods.[1] These media are used in the membrane filter technique for the isolation of fecal streptococcus and enterococcus groups.[1] This procedure can be used to test marine and fresh water sources.

m E Agar with the addition of 0.075% indoxyl-β-D-glucoside (m EI Agar) is recommended by the USEPA as a one-step procedure for the isolation and identification of enterococci in recreational water.[9] This method is used in the USEPA Beaches Environmental Assessment Closure and Health (BEACH) Program. The use of m EI Agar eliminates the necessity of transferring the incubated membrane to Esculin Iron Agar.

Principles of the Procedure

m E Agar is a highly selective and differential primary isolation medium that supports good growth of enterococci. Peptone and yeast extract provide carbon, nitrogen, minerals, vitamins and other growth factors for organism growth. Sodium chloride maintains the osmotic balance of the medium. Nalidixic acid and sodium azide act as selective agents to inhibit gram-negative bacteria. Cycloheximide inhibits fungi. At the concentration in the formula, 2,3,5-triphenyltetrazolium chloride (TTC) dyes enterococci colonies. TTC slightly inhibits growth of other microorganisms. In addition, the elevated incubation temperature of 41°C inhibits some indigenous microbial flora. Esculin is hydrolyzed by enterococci to form esculetin and dextrose. The esculetin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a black to reddish brown complex that appears in the medium surrounding the colonies. The production of black to

User Quality Control

Identity Specifications

Difco™ m E Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	7.12% solution, soluble in purified water upon boiling. Solution is light to medium amber with bluish cast, very slightly opalescent.
Prepared Appearance:	Light to medium amber with blue cast, slightly opalescent.
Reaction of 7.12% Solution at 25°C:	pH 7.1 ± 0.2

Difco™ Esculin Iron Agar

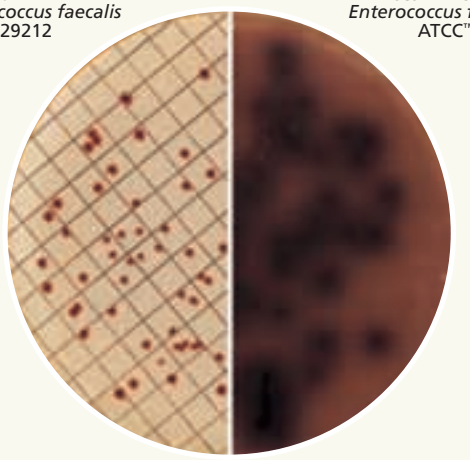
Dehydrated Appearance:	Tan to dark tan, free-flowing, homogeneous.
Solution:	1.65% solution, soluble in purified water upon boiling. Solution is medium amber with bluish cast, very slightly opalescent.
Prepared Appearance:	Medium amber with blue cast, slightly opalescent.
Reaction of 1.65% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Difco™ m E Agar and Difco™ Esculin Iron Agar

Prepare m E Agar per label directions and pour into 9 x 50 mm plates. Dilute the test organisms and filter through membrane filters. Place the filters on m E Agar plates and incubate the plates in an upright position for 48 hours at 41 ± 0.5°C. Remove the filters and place over prepared Esculin Iron Agar plates. After 20 minutes of incubation at 41 ± 0.5°C, count colonies giving positive esculin reaction (formation of black or reddish brown precipitate).

ORGANISM	ATCC™	INOCULUM CFU/10 mL	RECOVERY ON M E AGAR	REACTION ON ESCULIN IRON AGAR
Enterococcus faecalis	29212	20-60	Good/pink to red colonies	Black or reddish brown ppt
Enterococcus faecalis	33186	20-60	Good/pink to red colonies	Black or reddish brown ppt
Escherichia coli	25922	20-60	Marked to complete inhibition	None



reddish brown complex verifies the colonies as enteroccci and facilitates their enumeration. Agar is the solidifying agent.

Formulae

Difco™ m E Agar

Approximate Formula* Per Liter		
Yeast Extract	30.0	g
Peptone	10.0	g
Sodium Chloride	15.0	g
Esculin	1.0	g
Cycloheximide	0.05	g
Sodium Azide	0.15	g
Agar	15.0	g

Difco™ Esculin Iron Agar

Approximate Formula* Per Liter		
Esculin	1.0	g
Ferric Ammonium Citrate	0.5	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ m E Agar

1. Suspend 7.12 g of the powder in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45°C.
4. Add 0.024 g of nalidixic acid and 1.5 mL TTC Solution 1% (0.015 g Triphenyl Tetrazolium Chloride). Adjust to pH 7.1 if necessary.
5. Dispense 4-6 mL into 9 × 50 mm Petri dishes.
6. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Nalidixic acid is soluble in water with an alkaline pH.

Difco™ Esculin Iron Agar

1. Suspend 1.65 g of the powder in 100 mL of purified water (16.5 g in 1 L of purified water). Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45°C.
4. Dispense 4-6 mL into 9 × 50 mm Petri dishes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Follow the membrane filter procedure described in *Standard Methods for the Examination of Water and Wastewater*.[1]
2. Choose a sample size so that 20-60 colonies will result.
3. Place the filter on an m E Agar plate and incubate for 48 hours at 41 ± 0.5°C.
4. After incubation, remove the filter from m E Agar and place on an Esculin Iron Agar plate.
5. Incubate Esculin Iron Agar at 41 ± 0.5°C for 20 minutes.

Expected Results[1]

Pink to red enterococci develop a black or reddish-brown precipitate on the underside of the filter. Count colonies using a fluorescent lamp and a magnifying lens. Report results as estimated number or organisms per 100 mL of water.

Limitations of the Procedure

1. m E Agar and Esculin Iron Agar should be used in sequence.
2. Incubation at 41 ± 0.5°C is recommended.
3. Approximately 10% false-positive esculin reactions may be expected. When used as m EI Agar, USEPA reports a 6.0% false positive and 6.5% false negative rate with m E Agar.

References

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2. Slanetz and Bartley. 1957. J. Bacteriol. *74*:591.
3. American Society for Testing and Materials. 1996. Annual book of ASTM standards. Section 11, Water and environmental technology. PCN: 01-110296-16. ASTM, West Conshohocken, Pa.
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8. Dufour. 1983. Health effects criteria for fresh recreational waters. U.S. Environmental Protection Agency. Cincinnati, Ohio.
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Availability

Difco™ m E Agar

EPA SMWW

Cat. No.		
	233310	Dehydrated – 100 g
	233320	Dehydrated – 500 g

Difco™ Esculin Iron Agar

EPA SMWW

Cat. No.		
	248810	Dehydrated – 100 g

Difco™ TTC Solution 1%

EPA SMWW

Cat. No.		
	231121	Tube – 30 mL*

**Store at 2-8°C.*

EC Medium

Intended Use

EC Medium is a culture medium for the detection of coliform bacteria at 35°C and of *Escherichia coli* at an elevated temperature (44.5 or 45.5°C).

Summary and Explanation

EC Medium was devised by Hajna and Perry[1] and is used for the examination of water, milk, shellfish and other material for evidence of fecal pollution. Tennant et al. reported on the use of this medium for the estimation of *E. coli* densities in seawater and shellfish.[2] Fishbein and Surkiewicz used the EC confirmation test for recovery of *E. coli* from frozen foods and nut meats and reported that the test worked optimally when conducted at 45.5°C with incubation being limited to 24 hours.[3]

EC Medium is recommended for use in the fecal coliform Most Probable Number (MPN) procedure for the examination of water, wastewater and foods.[4,5] The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or nonfecal) when used as a confirmatory test.[6] It should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of fecal coliforms is required.

Principles of the Procedure

EC Medium contains peptone as a source of nutrients. Lactose provides fermentable carbohydrate for the growth of coliforms. Bile salts are inhibitory for gram-positive bacteria, particularly bacilli and fecal streptococci. The medium has a strong potassium phosphate buffering system to control the pH in the presence of considerable fermentative action. Sodium chloride maintains the osmotic balance of the medium.

Formula

Difco™ EC Medium

Approximate Formula* Per Liter		
Tryptose	20.0	g
Lactose	5.0	g
Bile Salts No. 3	1.5	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.5	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 37 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Refer to the various compendia for the specific procedures employing EC Medium.[4-8]

Expected Results

Refer to the compendia for the results expected when using this medium for the detection of coliforms and *E. coli*.[4-8]

Limitation of the Procedure

False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents.[9]

User Quality Control

Identity Specifications

Difco™ EC Medium

Dehydrated Appearance:	Light beige, freeflowing,homogeneous.
Solution:	3.7% solution, soluble in purified water upon warming. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 3.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ EC Medium

Prepare the medium per label directions. Inoculate and incubate tubes with fermentation vials at 44.5 ± 0.2°C for 24 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS
Enterococcus faecalis	19433	10^3	Inhibition	–
Escherichia coli	25922	10^3	Good	+
Escherichia coli	8739	10^3	Good	+

Uninoculated Tube

Escherichia coli ATCC™ 25922

References

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2. Tennant, Reid, Rockwell and Bynoe. 1961. Can. J. Microbiol. *1*:733.
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Availability

Difco™ EC Medium

AOAC | BAM | CCAM | COMPF | EPA | ISO | SMD | SMWW

Cat No.	231420	Dehydrated – 100 g
	231430	Dehydrated – 500 g
	231410	Dehydrated – 10 kg

EC Medium with MUG

Intended Use

EC Medium with MUG is used for detecting *Escherichia coli* in water, food and milk.

Summary and Explanation

EC Medium was developed by Hajna and Perry[1] to improve the methods for the detection of coliforms and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts No. 3. Growth of sporeformers and fecal streptococci is inhibited by the bile salts, while growth of *E. coli* is enhanced. EC Medium with MUG is the same formula as EC Medium with the addition of 4 methyl-umbelliferyl-β-D-glucuronide.

Feng and Hartman[2] developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) into Lauryl Tryptose Broth at a final concentration of 100 µg/mL. Robison[3] compared the fluorogenic assay with present methodology and found that total agreement between the two methods was 94.8%. Moburg[4] determined the amount of MUG could be reduced to a final concentration of 50 µg/mL without adversely affecting results. Koburger and Miller[5] recommended the incorporation of MUG into EC Broth for use in testing shellfish.

EC Medium with MUG is prepared according to the formula specified by the U.S. Environmental Protection Agency[6] and standard methods for water and food testing.[7,8]

Principles of the Procedure

Peptone provides the nitrogen, vitamins and amino acids in EC Medium with MUG. Lactose is the carbon source in this medium. Bile Salts No. 3 is the selective agent against gram-positive bacteria, particularly bacilli and fecal streptococci. Dipotassium phosphate and monopotassium phosphate are buffering agents. Sodium chloride maintains the osmotic balance of the medium.

E. coli produces the enzyme glucuronidase that hydrolyzes MUG to yield a fluorogenic product that is detectable under long wave (366 nm) UV light. The addition of MUG to EC Medium provides another criterion, in addition to growth response and gas production, to determine the presence of *E. coli* in food and environmental samples.

E

User Quality Control

Identity Specifications

Difco™ EC Medium with MUG

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.71% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 3.71% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ EC Medium with MUG

Prepare the medium per label directions. Inoculate tubes in duplicate with fresh 18-24 hour cultures. Incubate the first set at 35 ± 2°C for 24 ± 2 hours and the second set at 44.5 ± 0.2°C for 24 ± 2 hours. Read fluorescence under a long-wave UV light.

ORGANISM	ATCC™	RECOVERY AT 35°C/GAS	RECOVERY AT 44.5°C/GAS	FLUORESCENCE
Enterobacter aerogenes	13048	Good/±	Inhibition to good/–	–
Enterococcus faecalis	19433	Inhibition/–	Inhibition to good/–	–
Escherichia coli	25922	Good/+	Good/+	+

Escherichia coli
ATCC™ 25922

Formula

Difco™ EC Medium with MUG

Approximate Formula* Per Liter		
Tryptose	20.0	g
Lactose	5.0	g
Bile Salts No. 3	1.5	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.5	g
Sodium Chloride	5.0	g
MUG (4-methylumbelliferyl-β-D-glucuronide)	0.05	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 37.1 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense into test tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Follow the methods and procedures as stated in appropriate references.[6-8]

Expected Results

Following incubation, observe tubes for growth, production of gas and fluorescence. Positive gas production is demonstrated by displacement of the medium from the fermentation vial. Positive MUG reactions exhibit a bluish fluorescence under long-wave (approximately 366 nm) UV light. Typical strains of *E. coli* are positive for both gas production and fluorescence. Non-*E. coli* coliforms that grow may exhibit fluorescence but will not produce gas.

Strains of *Salmonella*, *Shigella* and *Yersinia* that produce glucuronidase may be encountered. These strains must be distinguished from *E. coli* on the basis of other parameters; i.e., gas production, growth at 44.5°C.

Limitations of the Procedure

1. Strains of *E. coli* that fail to grow in EC Medium with MUG, fail to produce gas, or fail to produce glucuronidase may infrequently be encountered.
2. The presence of endogenous glucuronidase in shellfish samples may cause false positive fluorescent reactions at the presumptive stage. To prevent this problem, the use of EC Medium with MUG in the confirmatory stage has been recommended.[5]

References

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Availability

Difco™ EC Medium with MUG

BAM CCAM EPA SMWW

Cat. No.	222100	Dehydrated – 100 g
	222200	Dehydrated – 500 g

EC Medium, Modified Novobiocin Antimicrobic Supplement

Intended Use

EC Medium, Modified is used with Novobiocin Antimicrobic Supplement in the detection of *Escherichia coli* O157:H7 in meat and poultry products.

Summary and Explanation

EC Medium, Modified and Novobiocin Antimicrobic Supplement are based on the formula for modified EC broth with novobiocin (mEC+n) as described by Okrend and Rose.[1] In modifying the EC Medium formula, Okrend and Rose reduced the Bile Salts No. 3 from 1.5 g per liter to 1.12 g per liter and added 20 mg per liter of sodium novobiocin. Okrend et al. reported that mEC+n was useful in the enrichment and detection of *E. coli* O157:H7 from meats and poultry products.[2-4]

Principles of the Procedure

Peptone supports good growth of *E. coli* O157:H7 and is rich in peptides and nitrogen. Lactose is an additional source of carbon for organisms, such as *E. coli*, that can ferment this sugar. Dipotassium phosphate and monopotassium phosphate are buffers that facilitate recovery of injured cells. Sodium chloride provides a suitable ionic environment for growth of microorganisms.

Selectivity of the medium is achieved by the incorporation of Bile Salts No. 3 into the base medium and by the addition of sodium novobiocin to the complete medium. These agents suppress the growth of nuisance organisms commonly found in foods. The sodium novobiocin is provided in the freeze-dried state as Novobiocin Antimicrobic Supplement. This supplement is rehydrated before use with sterile purified water.

User Quality Control

Identity Specifications

Difco™ EC Medium, Modified

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.66% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.66% Solution at 25°C:	pH 6.9 ± 0.2

Difco™ Novobiocin Antimicrobic Supplement

Lyophilized Appearance:	White cake.
Rehydrated Appearance:	Colorless solution.

Cultural Response

Difco™ EC Medium, Modified

Prepare the medium (without added novobiocin) per label directions. Inoculate and incubate at 35 ± 2°C for a maximum of 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	33186	10^3	None to poor
Escherichia coli O157:H7	35150	10-10^2	Good

Formulae

Difco™ EC Medium, Modified

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0	g
Bile Salts No. 3	1.12	g
Lactose	5.0	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.5	g
Sodium Chloride	5.0	g

Difco™ Novobiocin Antimicrobic Supplement

Formula Per 10 mL Vial

Sodium Novobiocin	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ EC Medium, Modified

1. Dissolve 36.6 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.
3. Aseptically add 10 mL rehydrated Novobiocin Antimicrobic Supplement. Mix well.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Novobiocin Antimicrobic Supplement

1. Aseptically add 10 mL sterile purified water to the vial.
2. Shake to dissolve the contents.

Procedure

Many procedures and systems have been described for the use of mEC+n in the selective and differential enrichment of *E. coli* O157:H7 in meat and poultry samples. Consult appropriate references.[4-6] The procedure for the enrichment and detection of *E. coli* O157:H7 in meat and poultry samples using mEC+n described here was in use by the USDA.[2-4] More recently, the USDA has replaced mEC+n with modified TSB with novobiocin plus casamino acids (mTSB+n).

1. Inoculate 25 g of meat sample into 225 mL of EC Medium, Modified, with novobiocin in a stomacher bag. Blend or stomach as required (i.e., 2 minutes) for thorough mixing.
2. Incubate at 35°C for 24 hours.
3. Dilute cultures 10-fold in Butterfield's Phosphate Diluent and inoculate 0.1 mL of appropriate dilutions using a spread plate technique onto MacConkey Sorbitol Agar (MSA) and MacConkey Sorbitol Agar with BCIG (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) agar plates.
4. Incubate plates at 42°C for 24 hours.
5. Examine MSA plates for sorbitol-negative colonies (white) and MSA-BCIG plates for sorbitol-negative, BCIG-negative colonies (white).
6. Subculture sorbitol-negative colonies to respective plates of EMB Agar and Phenol Red Sorbitol (PRS) Agar containing MUG (4-methylumbelliferyl-β-D-glucuronide).
7. Incubate EMB and PRS-MUG Agar plates at 35°C for 18-24 hours. Examine plates for sorbitol fermentation, MUG reaction (fluorescence), and typical *E. coli* growth on EMB Agar.

Expected Results

Growth in EC Medium, Modified, with novobiocin is demonstrated as an increase in turbidity. Colonies of *E. coli* O157:H7 appear white on MacConkey Sorbitol and MacConkey Sorbitol-BCIG Agars. Fermentation of sorbitol in Phenol Red Sorbitol Broth is demonstrated by the production of a yellow color in the medium. With sorbitol nonfermenters, the color of the medium remains red to reddish purple. Positive MUG reactions are demonstrated as a blue fluorescence in the medium under long-wave UV light. Colonies of *E. coli* on EMB Agar appear blue-black to dark purple. A green metallic sheen may also be present.

Cultures that are sorbitol-negative, MUG-negative and produce blue-black to dark purple colonies with a green metallic sheen on EMB Agar are indicative of *E. coli* O157:H7. These cultures should be tested serologically and with additional biochemical testing to confirm their identity as *E. coli* O157:H7.

References

1. Okrend and Rose. 1989. Isolation and identification of *E. coli* O157:H7 from meat. USDA Food Safety Inspection Service. Rev. 3 of Laboratory Communication no. 38. *E. coli* O157:H7. 20 December 1989. U.S. Department of Agriculture, Washington, D.C.
2. Okrend, Rose and Bennett. 1990. J. Food Prot. *53*:249.
3. Okrend, Rose and Lattuada. 1990. J. Food Prot. *53*:941.
4. Okrend, Rose and Matner. 1990. J. Food Prot. *53*:936.
5. Hawkins and Orme. 1995. Proc. West. Sec., Amer. Soc. Animal Sci. vol. 46.
6. Johnson, Durham, Johnson and MacDonald. 1995. Appl. Environ. Microbiol. *61*:386.

Availability

Difco™ EC Medium, Modified

CCAM COMPF

Cat. No.	234020	Dehydrated – 500 g
	234010	Dehydrated – 2 kg
	234001	Dehydrated – 10 kg

Difco™ Novobiocin Antimicrobic Supplement

CCAM COMPF

Cat. No.	231971	Vial – 6 × 10 mL*

**Store at 2-8°C.*

EE Broth Mossel Enrichment

Intended Use

EE Broth Mossel Enrichment is used for selectively enriching and detecting *Enterobacteriaceae*, particularly from foods.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

EE Broth Mossel Enrichment is prepared according to the formula of Mossel, Visser and Cornelissen.[4] The formula contains dextrose to facilitate growth of most *Enterobacteriaceae*, thus ensuring the detection of *Salmonella* and other lactose-negative organisms. EE Broth Mossel Enrichment should be used as an enrichment broth, followed by a selective medium; e.g., Violet Red Bile Agar.

The enumeration of *Enterobacteriaceae* is of great concern in monitoring the sanitary condition of food. *Enterobacteriaceae* can be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers.[5] Recovery relies on proper resuscitation of damaged cells. EE Broth Mossel Enrichment is used to detect and enumerate *Enterobacteriaceae* found per milliliter or per gram of test sample of food when performing the Most Probable Number (MPN) technique with pre-enrichment.[6,7]

EE Broth Mossel Enrichment is listed in the *USP* as one of the recommended media for the isolation of bile-tolerant gram-negative bacteria from nonsterile pharmaceutical products.[1]

Principles of the Procedure

Peptones provide nitrogen, vitamins and amino acids. Dextrose is a carbon source. Disodium phosphate and monopotassium phosphate are buffering agents. Brilliant green and oxgall are selective agents.

Formulae

Difco™ EE Broth Mossel Enrichment

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	10.0	g
Dextrose	5.0	g
Disodium Phosphate	8.0	g
Monopotassium Phosphate	2.0	g
Brilliant Green	15.0	mg
Oxgall	20.0	g

BBL™ EE Broth Mossel Enrichment

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	10.0	g
Dextrose	5.0	g
Oxgall	20.0	g
Disodium Phosphate	8.0	g
Monopotassium Phosphate	2.0	g
Brilliant Green	15.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water.
2. Heat with frequent agitation until dissolved. DO NOT OVERHEAT. Media is heat sensitive.
3. Dispense into tubes or bottles as required.
4. Heat at 100°C in water bath or flowing steam for 30 minutes. DO NOT AUTOCLAVE.
5. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[6,7]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For food samples, refer to appropriate standard references for details on test methods for performing MPN technique with enrichment using EE Broth Mossel Enrichment.[6,7]

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *Enterobacteriaceae* using EE Broth Mossel Enrichment.[1]

Expected Results

Acid production causes the color of EE Broth Mossel Enrichment to become yellow. A negative reaction results in no color change and the medium remains green.

Uninoculated Tube

Escherichia coli ATCC™ 25922

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ EE Broth Mossel Enrichment

Dehydrated Appearance:	Light green, free flowing, homogeneous.
Solution:	4.5% solution, soluble in purified water. Solution is emerald green, clear.
Prepared Appearance:	Emerald green, clear.
Reaction of 4.5% Solution at 25°C:	7.2 ± 0.2

Cultural Response

Difco™ EE Broth Mossel Enrichment

Prepare the medium per label directions. Inoculate 9 mL tubes and incubate at 35 ± 2°C for 18-24 hours and 48 hours, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID
Enterobacter aerogenes	13048	30-100	Good	+ (yellow)
Escherichia coli	25922	30-100	Good	+ (yellow)
Shigella boydii	12030	30-100	Good	–
Staphylococcus aureus	25923	30-100	Marked to complete inhibition	–

Inoculate 100 mL bottles and incubate at 30-35°C for 18-24 hours and 48 hours, if necessary. Inoculate a 20 mL tube with *Escherichia coli* ATCC 8739 and incubate at 35-37°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY
Escherichia coli	8739	<100	30-35°C	24	Growth
Escherichia coli	8739	<100	35-37°C	18-48	Growth
Pseudomonas aeruginosa	9027	<100	30-35°C	24	Growth
Staphylococcus aureus	6538	>100	30-35°C	48	No growth

Identity Specifications

BBL™ EE Broth Mossel Enrichment

Dehydrated Appearance:	Fine, homogeneous and free of extraneous material.
Solution:	4.5% solution, soluble in purified water. Solution is medium to dark green with or without a tint of yellow or blue; clear to slightly hazy.
Prepared Appearance:	Medium to dark green with or without a tint of yellow or blue; clear to slightly hazy.
Reaction of 4.5% Solution at 25°C:	pH 7.2 ± 0.2

BBL™ EE Broth Mossel Enrichment (prepared)

Appearance:	Medium to dark green and clear to trace hazy.
Reaction at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ EE Broth Mossel Enrichment

Prepare the medium per label directions. Inoculate 10 mL tubes and incubate at 35 ± 2°C for 18-24 hours and 48 hours, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID
Escherichia coli	25922	10^3-10^4	Good	+ (yellow)
Pseudomonas aeruginosa	10145	10^3-10^4	Good	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	+ (yellow)
Shigella sonnei	9290	10^3-10^4	Good	– to reduced (yellow green)

Inoculate 100 mL bottles and incubate at 30-35°C for 18-24 hours and 48 hours, if necessary. Inoculate a 20 mL tube with *Escherichia coli* ATCC 8739 and incubate at 35-37°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY
Escherichia coli	8739	<100	30-35°C	24	Growth
Escherichia coli	8739	<100	35-37°C	18-48	Growth
Pseudomonas aeruginosa	9027	<100	30-35°C	24	Growth
Staphylococcus aureus	6538	>100	30-35°C	48	No growth

BBL™ EE Broth Mossel Enrichment (prepared)

Inoculate 90 mL bottles and incubate as directed below.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY	ACID
Enterobacter aerogenes	13048	10^2-10^3	35-37°C	18-48	Growth	+ (yellow)
Escherichia coli	25922	10^2-10^3	35-37°C	18-48	Growth	+ (yellow)
Salmonella enterica subsp. *enterica* serotype Typhimurium	13311	10^2-10^3	35-37°C	18-48	Growth	+ (yellow)
Escherichia coli	8739	10-100	30-35°C	18-24	Growth	+ (yellow) to – or weak
Pseudomonas aeruginosa	9027	10-100	30-35°C	18-24	Growth	N/A
Staphylococcus aureus	6538	10^2-10^3	30-35°C	48	No growth	–

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
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7. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ EE Broth Mossel Enrichment

COMPF EP ISO JP USP

Cat. No. 256620 Dehydrated – 500 g†

BBL™ EE Broth Mossel Enrichment

COMPF EP ISO JP USP

Cat. No. 297005 Dehydrated – 500 g†
292627 Prepared Bottles, 90 mL (wide mouth) – Pkg. of 10†

† QC testing performed according to USP/EP/JP performance specifications.

mEI Agar

Intended Use

mEI Agar is a selective culture medium used for the chromogenic detection and enumeration of enterococci in water by the single-step membrane filtration technique. It conforms with U.S. Environmental Protection Agency (USEPA) Approved Method 1600: *Enterococci in Water by Membrane Filtration Using membrane*-Enterococcus *Indoxyl*-β-*D-Glucoside Agar (mEI)*.

Summary and Explanation

Enterococci are found in the feces of humans and other warm-blooded animals. Although some strains are ubiquitous and are not related to fecal pollution, the presence of enterococci in water is an indication of fecal pollution and the possible presence of enteric pathogens.[1] In epidemiological studies conducted by the USEPA, it was found that the presence of enterococci had a higher correlation with swimming-associated gastroenteritis in fresh and marine water environments than fecal coliforms.[2] In 1986, the USEPA recommended that both *Escherichia coli* and enterococci be used as bacterial water quality indicators to monitor recreational waters.[3]

A two-step membrane filter (MF) method[4] was developed by Levin et al. to measure enterococci in fresh and marine recreational waters. Using mE agar, the method required a 48-hour incubation and a transfer of the membrane to another substrate medium, Esculin Iron Agar, to differentiate enterococci.

In 1997, the USEPA improved on the mE agar formulation by reducing the triphenyltetrazolium chloride component and adding the chromogen, indoxyl-β-D-glucoside. The new medium, mEI Agar,[1,5] was developed as a single-step procedure that does not require the transfer of the membrane filter to another substrate. Observation of a blue halo around colonies in 24 hours is confirmatory for the presence of enterococci. A wide range of sample volumes or dilutions can

User Quality Control

Identity Specifications

Difco™ mEI Agar

Dehydrated Appearance:	Light to medium beige, free-flowing, homogeneous.
Solution:	7.2% solution, soluble in purified water upon boiling. Solution is medium to dark amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 7.2% Solution at 25°C:	pH 7.1 ± 0.2

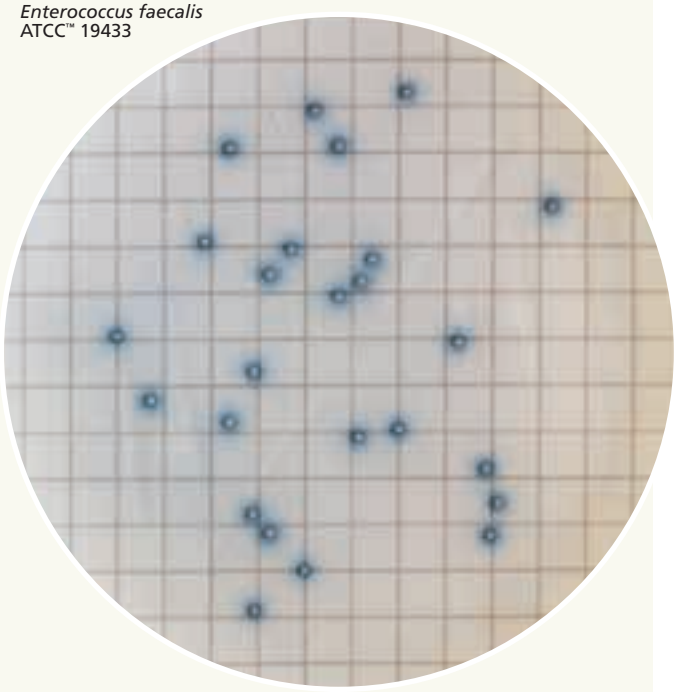
Cultural Response

Difco™ mEI Agar

Prepare the medium per label directions. Inoculate and incubate at 41± 0.5°C for 24 ± 2 hours. Count all colonies with blue halos.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
Enterococcus faecalis	19433	20-80	Good	Blue halo
Enterococcus faecium	19434	20-80	Good	Blue halo
Escherichia coli	25922	20-80	Marked to complete inhibition	–

Enterococcus faecalis
ATCC™ 19433



be tested by this single-step MF procedure for the detection and enumeration of enterococci in potable, fresh, estuarine, marine and shellfish-growing waters.

BD mEI Agar conforms to the 1986 revisions to the bacteriological ambient water quality criteria, that included the indicator bacteria *E. coli* and enterococci, which provide better correlation with swimming-associated gastrointestinal illness. In response to this health risk, the USEPA established the Beaches Environmental Assessment Closure and Health (Beach) Program. This method is published for use in the Beach Program.[5]

The USEPA published false-positive rate is 6.0% and false-negative rate is 6.5%.[5] Colonies having a blue halo can be verified as enterococci by appropriate biochemical procedures in instances where required in evidence gathering or for performing quality control for the initial use of the test.[5]

Principles of the Procedure

mEI Agar contains peptone that supplies nitrogen and carbon compounds. Sodium chloride maintains osmotic equilibrium. Esculin is hydrolyzed by enterococci to form esculetin and dextrose. Cycloheximide inhibits fungi. Sodium azide acts as a selective agent to inhibit gram-negative bacteria. Yeast extract provides trace elements, vitamins and amino acids. The addition of the chromogen indoxyl-β-D-glucoside results in the production of an insoluble indigo blue complex by β-D-glucosidase-positive enterococci, which diffuses into the surrounding medium, forming a blue halo around the colony.[6] Agar is incorporated into the medium as the solidifying agent.

Formula

Difco™ mEI Agar

Approximate Formula* Per Liter		
Peptone	10.0	g
Sodium Chloride	15.0	g
Esculin	1.0	g
Cycloheximide	0.05	g
Sodium Azide	0.15	g
Yeast Extract	30.0	g
Indoxyl-β-D-glucoside	0.75	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 72 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes and cool in a 50°C water bath.
4. Prepare a solution of 0.24 g of nalidixic acid in 5 mL of purified water. Add a few drops of 0.1 N NaOH to dissolve. Add this solution to 1 L of mEI medium.
5. Add 0.02 g of triphenyltetrazolium chloride separately to the mEI medium and mix well.
6. Dispense 5 mL amounts into 9 × 50 mm or 15 × 60 mm plates and allow to solidify.
7. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Collect and prepare water samples in accordance to recommended guidelines.[7,8]
2. Test sample volumes following the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.[7] Select sample volumes to produce 20-60 colonies on the membrane filter.
3. After sample has been filtered, aseptically remove membrane filter from filter base and roll it onto mEI Agar to avoid the formation of bubbles between the membrane and the agar surface.
4. Invert inoculated plates and incubate for 24 ± 2 hours at 41 ± 0.5°C.
5. After incubation, count and record the number of colonies with a blue halo using an illuminated lens with a 2-5× magnification.
6. Calculate and report the number of enterococci colonies per 100 mL of sample.

Expected Results

Colonies with a blue halo regardless of color may be presumptively identified as enterococci. Refer to the USEPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.[9]

Limitations of the Procedure

1. Choose a water sample size that will result in 20-60 colonies per filter.
2. Minimize the exposure of mEI Agar to light before and during incubation, as light may destroy the chromogen.
3. Overheating may cause darkening of the medium.[2]

References

1. U.S. Environmental Protection Agency. 1997. Method 1600: Membrane filter test method for enterococci in water. Publication EPA-821-R-97-004a. Office of Water, USEPA, Washington, D.C.
2. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: enterococci and *Escherichia coli*. Publication EPA/821/R-97/004. Office of Science and Technology, USEPA, Washington, D.C.
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4. Levin, Fischer and Cabelli. 1975. Appl. Microbiol. *30*:66.
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8. ASTM International. 2002. Annual book of ASTM standards. Water and environmental technology. ASTM International, West Conshohocken, Pa.
9. Bordner, Winter and Scarpino (ed.). 1978. Microbiological methods for monitoring the environment: water and wastes. Publication EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Availability

Difco™ mEI Agar

EPA SMWW

Cat. No.		
	214885	Dehydrated – 100 g
	214881	Dehydrated – 500 g

BBL™ mEI Agar

Cat. No.		
	215045	Prepared Plates – Pkg. of 20*
	215047	Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*

EVA Broth

Intended Use
EVA (Ethyl Violet Azide) Broth is used for detecting and confirming enterococci in water and other specimens as an indication of fecal contamination.

Summary and Explanation
The presence of enterococci in water and other specimens indicates fecal contamination. Mallmann and Seligmann[1] compared various enrichment media for detecting fecal streptococci and found that Azide Dextrose Broth presumptively identified the streptococci. However, because gram-positive bacteria other than enterococci grow in that medium, confirmation is necessary. Litsky et al.[2] studied various dyes and selective agents and formulated a medium using ethyl violet and sodium azide as selective agents. The medium known as Ethyl Violet Azide (EVA) Broth is specific for enterococci. In conjunction with Azide Dextrose Broth, EVA Broth is used to confirm the presence of enterococci.

Principles of the Procedure
EVA Broth contains peptones as sources of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate. Sodium azide and ethyl violet inhibit gram-positive bacilli and gram-positive cocci other than enterococci. Monopotassium and dipotassium phosphates buffer the medium. Sodium chloride provides osmotic balance.

Formula
Difco™ EVA Broth

Approximate Formula* Per Liter

Proteose Peptone No. 3	8.0	g
Pancreatic Digest of Casein	12.0	g
Dextrose	5.0	g
Dipotassium Phosphate	2.7	g
Monopotassium Phosphate	2.7	g
Sodium Chloride	5.0	g
Sodium Azide	0.4	g
Ethyl Violet	0.83	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Dissolve 35.8 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
See appropriate references for specific procedures.

Expected Results
Growth of enterococci.

User Quality Control

Identity Specifications
Difco™ EVA Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.58% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 3.58% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response
Difco™ EVA Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	19433	10^2-10^3	Good
Enterococcus faecalis	29212	10^2-10^3	Good
Escherichia coli	25922	10^3	Inhibition

Uninoculated Tube | *Enterococcus faecalis* ATCC™ 29212

References
1. Mallmann and Seligmann. 1950. Am. J. Pub. Health *40*:286.
2. Litsky, Mallmann and Fifield. 1953. Am. J. Pub. Health *43*:873.

Availability
Difco™ EVA Broth

Cat. No. 212107 Dehydrated – 500 g

Egg Yolk Agar, Modified

Intended Use

Egg Yolk Agar, Modified is a differential medium used in the isolation and presumptive differentiation of *Clostridium* spp. And other obligately anaerobic bacilli.

Summary and Explanation

Egg Yolk Agar, Modified is based on an egg yolk medium developed by McClung and Toabe for the isolation and presumptive differentiation of clostridia based on lecithinase and lipase production and proteolytic activity.[1]

In this modification, CDC Anaerobe Agar (without vitamin K_1, which is subsequently supplied by egg yolks) is used as the basal medium instead of the McClung and Toabe formulation. CDC Anaerobe Agar is an enriched, nonselective medium that was developed at the Center for Disease Control (currently named the Centers for Disease Control and Prevention [CDC]) for use in the cultivation of obligately anaerobic microorganisms, particularly those found in clinical materials.[2]

CDC Anaerobe Agar is supplemented with egg yolk suspension for demonstration of lecithinase and lipase production and proteolytic activity.[1-7]

Principles of the Procedure

Enzymatic digests of casein and soybean meal supply amino acids and other complex nitrogenous substances. Yeast extract primarily provides the B-complex vitamins. Hemin improves the growth of anaerobic microorganisms. L-cystine is a reducing agent and an essential amino acid.

An egg yolk suspension is incorporated to detect the production of lecithinase and lipase and proteolytic activity. Lecithinase degrades the lecithin present in the egg yolks, producing an insoluble, opaque precipitate in the medium surrounding growth.

Lipase breaks down free fats present in the egg yolks, causing an iridescent, "oil on water" sheen to form on the surface of the colonies. Since the lipase reaction may be delayed, plates should be kept up to 7 days before regarding them as negative for lipase production.

Proteolysis is indicated by clear zones in the medium surrounding growth.

Procedure

This medium should be reduced immediately prior to inoculation by placing it under anaerobic conditions for 18-24 hours.[3] An efficient and convenient method for obtaining suitable anaerobic conditions is through the use of the **BD GasPak**™ EZ anaerobic system or an alternative anaerobic system.

Use standard procedures to obtain isolated colonies from specimens.

Clostridium perfringens
ATCC™ 12919

Inoculate an enrichment broth, such as Enriched Thioglycollate Medium, at the same time as the primary plates to detect small numbers of anaerobes.

Incubate plates and tubes immediately after inoculation, with plates in an inverted position (agar side up), under anaerobic conditions at 35°C, or place the media in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates and tubes is accumulated (no longer than 3 hours).[8] Incubate for at least 48 hours, and, if no growth occurs, continue incubation for up to 7 days.

Examine for growth after 48 hours of incubation. Cultures should not be regarded as negative until after 7 days of incubation.

Expected Results

After a minimum of 48 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Examine for lecithinase and lipase production and proteolytic activity. Plates with isolates negative for lipase should be held up to 7 days. Examine colonial morphology and a Gram stain of the organism to confirm presumptive identification.

References

1. McClung and Toabe. 1947. J. Bacteriol. *53*:139.
2. Dowell, Lombard, Thompson and Armfield. 1977. Media for isolation, characterization and identification of obligately anaerobic bacteria. CDC laboratory manual. Center for Disease Control, Atlanta, Ga.
3. Dowell and Hawkins. 1987. Laboratory methods in anaerobic bacteriology. CDC laboratory manual. HHS Publication No. (CDC) 87-8272. Centers for Disease Control, Atlanta, Ga.
4. Allen, Emery and Siders. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

5. Jousimies-Somer, Summanen and Finegold. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
6. Holdeman, Cato and Moore (ed.). 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, Va.
7. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
8. Martin. 1971. Appl. Microbiol. *22*:1168.

Availability

BBL™ Egg Yolk Agar, Modified

BS12 CMPH MCM9

Cat. No. 297873 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Elliker Broth

Intended Use

Elliker Broth, also known as Lactobacilli Broth, is used for cultivating streptococci and lactobacilli, particularly in dairy procedures.

Summary and Explanation

Testing for lactic acid bacteria in dairy products may be useful for various reasons.[1] These include determining the cause of acid defects in dairy products, evaluating lactic starter cultures and controlling the quality of cured cheese, cultured milks and uncultured products.[1] Lactic acid bacteria found in dairy products are primarily *Streptococcus*, *Lactococcus*, *Leuconostoc* and *Lactobacillus*.[1]

Elliker Broth is prepared according to the formulation of Elliker, Anderson and Hannesson,[2] and modified by McLaughlin.[3] This slightly acidic medium contains nutrients to support the growth of streptococci and lactobacilli.

A modification of Elliker Broth, Lactic (Elliker) Agar is recommended for general purpose enumeration of lactic acid bacteria.[1]

Principles of the Procedure

Peptone and gelatin provide the nitrogen and amino acids in Elliker Broth. Yeast extract is the vitamin source in this formula. Dextrose, lactose and saccharose are the fermentable carbohydrates. Sodium chloride maintains the osmotic balance of the medium, and ascorbic acid is added to create a proper environment for organism growth. Sodium acetate is a selective agent against gram-negative bacteria.

Formula

Difco™ Elliker Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	20.0	g
Yeast Extract	5.0	g
Gelatin	2.5	g
Dextrose	5.0	g
Lactose	5.0	g
Saccharose	5.0	g
Sodium Chloride	4.0	g
Sodium Acetate	1.5	g
Ascorbic Acid	0.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 48.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Elliker Broth

Dehydrated Appearance:	Light to medium beige, free-flowing, homogeneous.
Solution:	4.85% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 4.85% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Elliker Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours except *Streptococcus cremoris* which is incubated at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus casei	7469	10^2-10^3	Good
Lactobacillus delbrueckii subsp. *lactis*	8000	10^2-10^3	Good
Lactobacillus sp.	11506	10^2-10^3	Fair
Streptococcus cremoris	9596	10^2-10^3	Good

Procedure

For a complete discussion on the isolation and identification of streptococci and lactobacilli, refer to standard methods in food testing.[1,4-6]

Expected Results

Refer to appropriate references and procedures for results.

References

1. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products. 17th ed. American Public Health Association, Washington, D.C.
2. Elliker, Anderson and Hannesson. 1956. J. Dairy Sci. 39:1611.
3. McLaughlin. 1946. J. Bacteriol. *51*:560.
4. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th. ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Elliker Broth

SMD

Cat. No. 212183 Dehydrated – 500 g

Endo Agar

Intended Use

Endo Agar is a differential and slightly selective culture medium for the detection of coliform and other enteric microorganisms.

Summary and Explanation

The majority of the enteric plating media developed in the early years of the 20th century utilized either mixtures of bile salts or individual salts as selective agents to achieve inhibition of gram-positive species. In 1904, Endo reported the development of a culture medium for the differentiation of lactose fermenters from the nonfermenters in which no bile salts were used.[1] Inhibition of gram-positive microorganisms was achieved by the sodium sulfite and basic fuchsin contained in the formulation. Endo's Fuchsin Sulphite Infusion Agar was the original name for this medium,[2] which is known today as Endo Agar. It was developed initially in order to facilitate the isolation and identification of the typhoid bacillus.

The original formula has been modified extensively since its introduction. The meat infusions have been replaced by a peptic digest of animal tissue. The dye composition and concentration also have been adjusted.

Over the years, Endo Agar has been an important medium in the microbiological examination of potable water and wastewater, dairy products and foods; however, the current compendia of standard methods for the examination of these materials recommend alternative media formulations.[3-5]

Principles of the Procedure

The selectivity of Endo Agar is due to the sodium sulfite/basic fuchsin combination, which results in the suppression of gram-positive microorganisms. It is classified as only slightly selective since other media contain more potent inhibitors of the gram-positive microorganisms. Coliforms ferment the lactose, produce pink to rose-red colonies and similar coloration of the medium. The colonies of organisms that do not ferment lactose are colorless to faint against the pink background of the medium.

Formula

BBL™ Endo Agar

Approximate Formula* Per Liter		
Dipotassium Phosphate	3.5	g
Peptic Digest of Animal Tissue	10.0	g
Agar	15.0	g
Lactose	10.0	g
Sodium Sulfite	2.5	g
Basic Fuchsin	0.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

BBL™ Endo Agar

Dehydrated Appearance:	Fine, homogeneous powder that may contain a large amount of minute to small dark particles.
Solution:	4.15% solution, soluble in purified water upon boiling. Solution is light to medium, pink rose to tan rose trace orange, moderately hazy to hazy. May contain a moderate amount of small dark red particles and a large amount of minute dark red particles.
Prepared Appearance:	Light to medium, pink rose to tan rose trace orange, moderately hazy to hazy. May contain a moderate amount of small dark red particles and a large amount of minute dark red particles.
Reaction of 4.15% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

BBL™ Endo Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^4-10^5	Poor to fair	Pink to rose-red
Escherichia coli	25922	10^3-10^4	Good	Rose-red, green metallic sheen
Klebsiella pneumoniae	33495	10^3-10^4	Good	Pink to rose-red mucoid
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless to pale pink

Directions for Preparation from Dehydrated Product

1. Suspend 41.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C. Resuspend precipitate by gentle mixing before use. Endo Agar should be prepared as needed.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Expected Results

Typical colonial morphology on Endo Agar is as follows:

Escherichia coli	Pink to rose-red, green metallic sheen
Enterobacter/Klebsiella	Large, mucoid, pink
Proteus	Colorless to pale pink
Salmonella	Colorless to pale pink
Shigella	Colorless to pale pink
Pseudomonas	Irregular, colorless
Gram-positive bacteria	No growth to slight growth

References

1. Endo. 1904. Zentralbl. Bakteriol., Abt. 1, Orig. *35*:109.
2. Levin and Schoenlein. 1930. A compilation of culture media for the cultivation of microorganisms. Williams & Wilkins, Baltimore, Md.
3. Eaton, Rice and Baird (ed). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
4. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products. 17th ed. American Public Health Association, Washington, D.C.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

BBL™ Endo Agar

CCAM

Cat. No. 211199 Dehydrated – 500 g

United States and Canada

Cat. No. 221167 Prepared Plates – Pkg. of 20*
221265 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254016 Prepared Plates – Pkg. of 20*
254074 Prepared Plates – Ctn. of 120*

**Store at 2-8°C.*

m Endo Agar LES

Intended Use

m Endo Agar LES is used for enumerating coliforms in water by membrane filtration.

Summary and Explanation

McCarthy, Delaney and Grasso[1] formulated Endo Agar LES (Lawrence Experimental Station) for testing water for coliform bacteria by a two-step membrane filter procedure using Lauryl Tryptose Broth as a preliminary enrichment. They recovered higher numbers of coliforms by this method compared with the one step technique using m Endo Broth.

The American Public Health Association specifies using m Endo Agar LES in the standard total coliform membrane filtration procedure for testing drinking water[2] and bottled water.[3] It is also specified for use in the completed phase of the standard total coliform fermentation technique.[2] The coliform bacteria are bacteria that produce a red colony with a metallic (golden) sheen within 24 hours incubation at 35°C on an Endo-type medium.

Principles of the Procedure

m Endo Agar LES contains peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins, which stimulate bacterial growth. Lactose is the carbohydrate. Phosphates are buffering agents. Sodium chloride maintains the osmotic balance of the medium. Sodium desoxycholate and sodium lauryl sulfate are added as inhibitors. Basic fuchsin is a pH indicator. Sodium sulfite is added to decolorize the basic fuchsin solution. Agar is the solidifying agent.

Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulfite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism produces aldehydes with the rapid fermentation of lactose. If the inoculum is too heavy, the sheen will be suppressed. Lactose-nonfermenting bacteria form clear, colorless colonies.

Formula

Difco™ m Endo Agar LES

Approximate Formula* Per Liter

Yeast Extract	1.2	g
Casitone	3.7	g
Thiopeptone	3.7	g
Tryptose	7.5	g
Lactose	9.4	g
Dipotassium Phosphate	3.3	g
Monopotassium Phosphate	1.0	g
Sodium Chloride	3.7	g
Sodium Desoxycholate	0.1	g
Sodium Lauryl Sulfate	0.05	g
Sodium Sulfite	1.6	g
Basic Fuchsin	0.8	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 51 g of the powder in 1 L of purified water containing 20 mL of 95% ethanol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Place a membrane filter absorbent pad inside the cover of a Petri dish.
2. Add 1.8-2.0 mL Lauryl Tryptose Broth or Lauryl Sulfate Broth to each pad.
3. Run the water sample through a membrane filter.

4. Place the filter, top side up, onto the pad containing Lauryl Tryptose Broth or Lauryl Sulfate Broth. Use a rolling motion to avoid entrapping air bubbles.
5. Incubate at 35 ± 0.5°C for 1.5-2.5 hours. Transfer the membrane from the pad to the surface of the m Endo Agar LES medium in the Petri dish bottom, keeping the side on which the bacteria have been collected facing upward.
6. Leave the filter pad in the lid and incubate the plates in the inverted position at 35 ± 0.5°C for 22 ± 2 hours.
7. Observe and count all colonies that are red and have a metallic sheen.

Expected Results

All colonies that are red and have the characteristic metallic sheen are considered coliforms. The sheen may cover the entire colony, may only be in the center or may appear only around the edges.

Limitations of the Procedure

Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies (dark red or nucleated colonies without sheen). It is advisable to verify both colony types.[2]

References

1. McCarthy, Delaney and Grasso. 1961. Water Sewage Works *108*:238.
2. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
3. Kim and Feng. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ m Endo Agar LES

COMPF SMD SMWW

Cat. No.	273610	Dehydrated – 100 g
	273620	Dehydrated – 500 g

User Quality Control

Identity Specifications

Difco™ m Endo Agar LES

Dehydrated Appearance:	Purple, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in purified water containing 2% ethanol upon boiling. Solution is pinkish-red, slightly opalescent to opalescent with precipitate.
Prepared Appearance:	Rose colored, slightly opalescent, with precipitate.
Reaction of 5.1% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ m Endo Agar LES

Prepare the medium per label directions. Use the membrane filter technique to inoculate filters and preincubate on pads saturated with Lauryl Tryptose Broth or Lauryl Sulfate Broth at 35 ± 0.5°C for 1.5-2 hours. Transfer filters to plates of m Endo Agar LES and incubate at 35 ± 0.5°C for 22 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	30-80	Good	Red with sheen
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-80	Good	Pink
Staphylococcus aureus	25923	10^3	Marked to complete inhibition	–

E

m Endo Broth MF™

Intended Use

m Endo Broth MF™* is used for enumerating coliform organisms in water by membrane filtration.

**MF is a trademark of Millipore Corporation.*

Summary and Explanation

m Endo Broth MF is prepared according to the formulation of the Millipore Corporation[1] for selectively isolating coliform bacteria from water and other specimens using the membrane filtration technique. The medium is a combination of the former m HD Endo Medium and Lauryl Tryptose Broth.

The American Public Health Association (APHA) specifies using m Endo Broth MF in the standard total coliform membrane filtration procedure for testing water[2] and bottled water.[3] APHA also specifies using m Endo Broth MF in the delayed-incubation total coliform procedure by adding sodium benzoate to make m Endo preservative medium.[2] The coliform bacteria are defined as bacteria that produce a red colony with a metallic sheen within 24 hours incubation at 35°C on an Endo-type medium.

Principles of the Procedure

m Endo Broth MF contains peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins, which stimulate bacterial growth. Lactose is the carbohydrate. Phosphates are buffering agents. Sodium chloride maintains the osmotic balance of the medium. Sodium desoxycholate and sodium lauryl sulfate are added as inhibitors of gram-positive bacteria. Basic fuchsin is a pH indicator. Sodium sulfite is added to decolorize the basic fuchsin solution. The ethanol additive increases the antibacterial nature of the formulation.

Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulfite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism

produces aldehydes with the rapid fermentation of lactose. If the inoculum is too heavy, the sheen will be suppressed. Lactose-nonfermenting bacteria form clear, colorless colonies.

Formula

Difco™ m Endo Broth MF™

Approximate Formula* Per Liter

Yeast Extract	1.5 g
Casitone	5.0 g
Thiopeptone	5.0 g
Tryptose	10.0 g
Lactose	12.5 g
Sodium Desoxycholate	0.1 g
Dipotassium Phosphate	4.375 g
Monopotassium Phosphate	1.375 g
Sodium Chloride	5.0 g
Sodium Lauryl Sulfate	0.05 g
Sodium Sulfite	2.1 g
Basic Fuchsin	1.05 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 48 g of the powder in 1 L of purified water containing 20 mL nondenatured ethanol.
2. Heat to boiling but avoid over-heating. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

CAUTION: Protect from light. Use immediately.

Procedure

1. Place a membrane filter absorbent pad inside a sterile 60 mm Petri dish.
2. Add 1.8-2.0 mL m Endo Broth MF to each pad.
3. Filter the water sample through a membrane filter.
4. Place filter top side up on the pad using a rolling motion to avoid entrapping air bubbles.
5. Invert the dish and incubate for 22-24 hours at 35 ± 0.5°C.
6. Observe and count all colonies that are red and have a metallic sheen.

Expected Results

All colonies that are red and have the characteristic metallic sheen are considered coliforms. The sheen may cover the entire colony, may only be in the center or may appear only around the edges.

Limitations of the Procedure

Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies (dark red or nucleated colonies without sheen). It is advisable to verify both colony types.[2]

References

1. Fifield and Schaufus. 1958. J. Am. Water Works Assoc. *50*:193.
2. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
3. Kim and Feng. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

User Quality Control

Identity Specifications

Difco™ m Endo Broth MF™

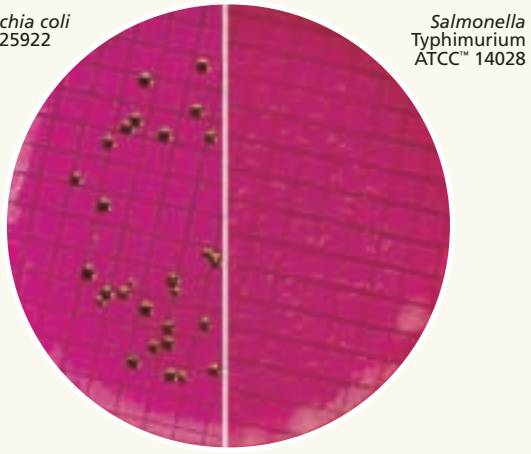
Dehydrated Appearance:	Pinkish purple, free-flowing, homogeneous.
Solution:	4.8% solution, partially soluble in purified water containing 2% ethanol upon boiling. Solution is pinkish-red, opalescent with precipitate.
Prepared Appearance:	Pinkish-red, opalescent with precipitate.
Reaction of 4.8% Solution at 25°C:	pH 7.2 ± 0.1

Cultural Response

Difco™ m Endo Broth MF™

Prepare the medium per label directions. Use the membrane filter technique to inoculate filters. Incubate on pads saturated with m Endo Broth MF at 35 ± 2°C for 24 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	20-80	Good	Red with green metallic sheen
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	20-80	Good	Colorless to pink
Staphylococcus aureus	25923	10^3-2×10^3	Marked inhibition	–



Availability

Difco™ m Endo Broth MF™

COMPF SMD SMWW

Cat. No.	274920	Dehydrated – 100 g
	274930	Dehydrated – 500 g

Enriched Thioglycollate Media

(See Thioglycollate Media)

Enteric Fermentation Base

Intended Use

Enteric Fermentation Base is used with added carbohydrate and indicator for differentiating microorganisms based on fermentation reactions.

Summary and Explanation

The fermentative properties of bacteria are valuable criteria in their identification.[1-4] A basal medium for determining the fermentation reactions of microorganisms must be capable of supporting growth of test organisms and be free from fermentable carbohydrates. Enteric Fermentation Base is prepared according to the formula described by Edwards and Ewing.[5]

Principles of the Procedure

Beef extract and peptone provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Sodium chloride maintains the osmotic balance of the medium. The microorganisms tested are differentiated by their ability to ferment a particular carbohydrate that has been added to the Enteric Fermentation Base. The fermentation and resultant acid production are indicated by a change in color of the pH indicator (Andrade's indicator) which is also added to the Enteric Fermentation Base.

Formula

Difco™ Enteric Fermentation Base

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	10.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 18 g of the powder in 1 L of purified water. Mix thoroughly.
2. Add 10 mL of Andrade's indicator.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45-50°C in a water bath.
6. Aseptically add 0.5% or 1% of sterile carbohydrate (see table).

User Quality Control

Identity Specifications

Difco™ Enteric Fermentation Base

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	1.8% solution, soluble in purified water. Solution is light amber, clear.
Prepared Medium (plain) + Andrade's Indicator:	Light pinkish-amber, clear.
Reaction of 1.8% Solution at 25°C:	pH 7.2 ± 0.1

Cultural Response

Difco™ Enteric Fermentation Base

Prepare the medium per label directions, without and with 1% dextrose. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-24 hours. Acid production is indicated by a change in color from light amber to dark pink or red. Check for gas production in at least 3% of the volume of the fermentation vial.

ORGANISM	ATCC™	RECOVERY	PLAIN ACID/GAS	w/ DEXTROSE ACID/GAS
Escherichia coli	25922	Good	–/–	+/+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	–/–	+/+
Shigella flexneri	12022	Good	–/–	+/–

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 with Dextrose | *Escherichia coli* ATCC™ 25922 Plain

CARBOHYDRATE	FINAL CONCENTRATION	ADD BEFORE AUTOCLAVING	ADD AFTER AUTOCLAVING
Adonitol	0.5%	X	–
Arabinose	0.5%	–	X
Cellobiose	0.5%	–	X
Dextrose (Glucose)	1%	X	–
Dulcitol	0.5%	X	–
Glycerol*	0.5%	X	–
Inositol	0.5%	X	–
Lactose	1%	–	X
Mannitol	1%	X	–
Salicin	0.5%	X	–
Sucrose	1%	–	X
Xylose	0.5%	–	X

**Medium containing glycerol should be autoclaved for 10 minutes at 15 lbs pressure (121°C).*

7. Dispense 9 mL amounts into test tubes containing inverted vials (Durham tubes).
8. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on identification of *Enterobacteriaceae*, refer to the appropriate procedures outlined in the references.[1-4,6]

Expected Results

A positive result for gas includes production in at least 3% of the volume of the fermentation tube. A positive reaction for acid is a change in color from light amber to dark pink or red.

Limitation of the Procedure

Negative tubes remain colorless and should be observed regularly for a total of 30 days.

References

1. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th edition. Mosby, Inc., St. Louis, Mo.
2. Murray, Baron, Jorgensen, Landry and Pfaller. (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Edwards and Ewing. 1972. Identification of *Enterobacteriaceae,* 3rd ed. Burgess Publishing Co., Minneapolis, Minn.
6. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology. Washington, D.C.

Availability

Difco™ Enteric Fermentation Base

Cat. No. 218281 Dehydrated – 500 g

Enterococcosel™ Agar • Enterococcosel™ Broth

Intended Use

Enterococcosel Agar, a Bile Esculin Agar with Azide, is used for the rapid, selective detection and enumeration of enterococci.[1]

Enterococcosel Broth, a Bile Esculin Broth with Azide, is recommended for use in the differentiation of enterococci and group D streptococci.

Summary and Explanation

Rochaix noted the value of esculin hydrolysis in the identification of enterococci.[2] The enterococci were able to split esculin, but other streptococci could not. Meyer and Schonfeld incorporated bile into the esculin medium and showed that 61 of 62 enterococci were able to grow and split esculin, whereas the other streptococci could not.[3] Swan used an esculin medium containing 40% bile salts and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.[4] Facklam and Moody preformed a comparative study of tests used to presumptively identify group D streptococci and found that the bile-esculin test provides a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.[5] According to current nomenclature, the group D antigen is considered non-specific since it is produced by the genera *Enterococcus*, *Pediococcus* and by certain streptococci.[6]

Isenberg et al. modified the Bile Esculin Agar formulation by reducing the bile concentration from 40 to 10 g/L and by adding sodium azide.[7] This modification is supplied as **Enterococcosel** Agar. Consult the text for a list of specimens for which this medium is recommended for primary isolation.[8]

Enterococcosel Broth has the same formula as **Enterococcosel** Agar with the agar omitted. Colonies suspected of being *Enterococcus faecalis* can be emulsified in 1 or 2 mL of broth and incubated at 35°C. The combination of esculin and a rather low concentration of bile in the presence of azide permits the selection and differentiation of enterococci by esculin hydrolysis (blackening of the medium) within 2 hours.[7]

Principles of the Procedure

Enterococci and Group D streptococci hydrolyze the glycoside esculin to esculetin and dextrose. Esculetin reacts with an iron salt, ferric ammonium citrate, to form a dark brown or black complex.[9] Oxgall is used to inhibit gram-positive bacteria other than enterococci. Sodium azide is inhibitory for gram-negative microorganisms.

User Quality Control

Identity Specifications

BBL™ Enterococcosel™ Agar

Dehydrated Appearance:	Medium fine, homogeneous, may contain some tan specks.
Solution:	5.6% solution, soluble in purified water upon boiling. Solution is medium, tan with a trace blue cast, clear to moderately hazy.
Prepared Appearance:	Medium, tan with a trace blue cast, clear to moderately hazy.
Reaction of 5.6% Solution at 25°C:	pH 7.1 ± 0.2

BBL™ Enterococcosel™ Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.3% solution, soluble in purified water upon heating. Solution is medium, yellow to tan to yellow green with a bluish cast, clear to hazy.
Prepared Appearance:	Medium, yellow to tan to yellow-green with a bluish cast, clear to hazy.
Reaction of 4.3% Solution at 25°C:	pH 7.1 ± 0.2

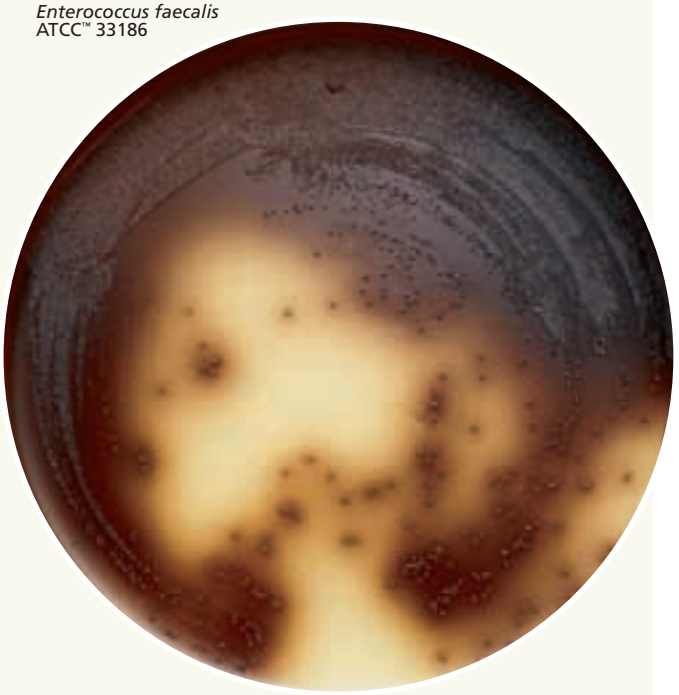
Cultural Response

BBL™ Enterococcosel™ Agar or Enterococcosel™ Broth

Prepare the medium per label directions. For agar, inoculate as described below. For broth, inoculate with fresh cultures. Incubate at 35 ± 2°C for 48 hours (agar) or 24 hours (broth).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AGAR	RECOVERY BROTH
Enterococcus faecalis	29212	10^3-10^4	Good, blackening	Good, blackening
Escherichia coli	25922	10^4-10^5	Complete inhibition	Partial to complete inhibition, no blackening
Streptococcus pyogenes	19615	10^4-10^5	Complete inhibition	Partial to complete inhibition, no blackening

Enterococcus faecalis
ATCC™ 33186



Formulae

BBL™ Enterococcosel™ Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Peptic Digest of Animal Tissue	3.0	g
Yeast Extract	5.0	g
Oxgall	10.0	g
Sodium Chloride	5.0	g
Esculin	1.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Azide	0.25	g
Sodium Citrate	1.0	g
Agar	13.5	g

BBL™ Enterococcosel™ Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   BBL™ Enterococcosel™ Agar – 56 g;
   BBL™ Enterococcosel™ Broth – 43 g.
   Mix thoroughly.
2. For agar, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. For broth, heat if necessary to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Agar

Use standard procedures to obtain isolated colonies from specimens. Incubate plates 24-48 hours at 35 ± 2°C in and aerobic atmosphere.

Broth

Colonies, from a primary isolation plate, suspected of being enterococci or group D streptococci can be emulsified in 2 mL of **Enterococcosel** Broth and incubated at 35 ± 2°C in an aerobic atmosphere.

Expected Results

After incubation, observe for typical growth and reaction:

Agar

Typical colonial morphology on **Enterococcosel** Agar is as follows:

Streptococci (non-group D)	No growth to trace growth.
Enterococci/group D streptococci	Small, but larger than group A streptococci. Translucent with brownish-black to black zones.
Staphylococci	Large, white, opaque.
Micrococci	Large, white, grayish.
Corynebacteria	Small to large, white to grayish-yellow, smooth and irregular.
Candida	Small to large, white.
Listeria monocytogenes	Small to large, translucent with brownish-black to black zones.
Gram-negative bacteria	No growth to trace growth.

Broth

Enterococci and group D streptococci turn the medium black within 2 hours when a heavy inoculum is used. Other organisms are inhibited or do not turn the medium black.

Limitations of the Procedure

Listeria monocytogenes, *Streptococcus bovis* group, *Pediococcus* and staphylococci may also grow on **Enterococcosel** Agar. However, staphylococci do not produce black zones. Other organisms (e.g., micrococci, *Candida*, corynebacteria and gram-negative bacteria) may appear as small colonies or produce trace growth.

References

1. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Rochaix. 1924. C.R. Soc. Biol. *90*:771.
3. Meyer and Schonfeld. 1926. Zentralbl. Bakteriol. Parasitenk. Infectionskr. Hyg. Abt. Orig. *99*:402.
4. Swan. 1954. J. Clin. Pathol. *7*:160.
5. Facklam and Moody. 1970. Appl. Microbiol. *20*:245.
6. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Isenberg, Goldberg and Sampson. 1970. Appl. Microbiol. *20*:433.
8. Cintron. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
9. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott William & Wilkins, Baltimore, Md.

Availability

BBL™ Enterococcosel™ Agar

CMPH2 ISO MCM9

Cat. No. 212205 Dehydrated – 500 g

United States and Canada

Cat. No. 221492 Prepared Plates – Pkg. of 20*
221493 Prepared Plates – Ctn. of 100*
221381 Prepared Slants – Pkg. of 10*
221382 Prepared Slants – Ctn. of 100*

Europe

Cat. No. 254019 Prepared Plates – Pkg. of 20*

BBL™ Enterococcosel™ Agar//Columbia CNA Agar, Modified with Sheep Blood

Cat. No. 297413 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ Enterococcosel™ Broth

Cat. No. 212207 Dehydrated – 500 g
221383 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*

Enterococcosel™ Agar with Vancomycin, 8 µg/mL

Intended Use

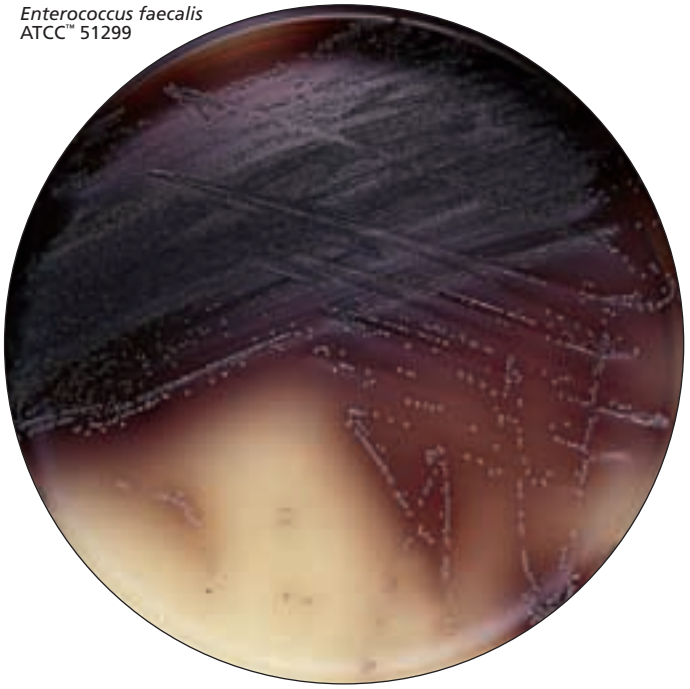
Enterococcosel Agar with Vancomycin, 8 µg/mL, is used for primary screening of asymptomatic gastrointestinal carriage of vancomycin-resistant enterococci (VRE).[1]

Summary and Explanation

Enterococci are known to cause a wide variety of infections. Most commonly they infect the urinary tract, abdomen, bloodstream, endocardium, biliary tract, burn wounds and in-dwelling catheters.[2] *Enterococcus faecalis* causes 80 to 90% of infections, while *E. faecium* causes the remainder.[3] Today the enterococci are the fourth leading cause of nosocomial infection and the third leading cause of bacteremia in the United States.[4] The case/fatality rates for enterococcal bacteria range from 12 to 68% with death due to sepsis in 4 to 50% of the cases.[5]

Because the potential exists for vancomycin-resistant genes to be transferred to other gram-positive organisms and because the treatment options for VRE infections are limited, the CDC issued infection control guidelines for hospitals and long-term

Enterococcus faecalis
ATCC™ 51299



care facilities.[6] Guidelines include stool and rectal swab culture surveys of asymptomatic patients who may be carrying VRE.

Principles of the Procedure

Enterococcosel Agar, a Bile Esculin Agar with Azide, is used for the rapid, selective detection and enumeration of enterococci.

Enterococci hydrolyze the glucoside esculin to esculetin and dextrose. Esculetin reacts with an iron salt to form a dark brown or black complex.[7] Ferric citrate is incorporated into the medium as an indicator of esculin hydrolysis and resulting esculetin formation. Oxgall is used to inhibit gram-positive bacteria other than enterococci. Sodium azide is inhibitory for gram-negative organisms.

Vancomycin at 8 µg/mL is used to detect resistance to vancomycin.[1,8]

Procedure

Allow the contents of a rectal swab (or a cotton-tipped swab sample obtained from a stool specimen) to elute in 1 mL of **Trypticase**™ Soy Broth.[1] Using a new swab, absorb eluent, rotate swab firmly several times against the upper inside wall of the tube to express excess fluid, roll the swab over a small area of the surface at the edge of the plate and streak from this inoculated area.

Incubate the plates in an inverted position (agar-side up) for 24-48 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Examine plates after 24 and 48 hours for the presence of translucent to light gray, pinpoint colonies exhibiting black halos (discoloration of the agar) in areas of heavy growth. Perform a Gram stain, catalase test and PYR test. Gram-positive cocci which are either catalase negative or weakly positive and PYR positive may be presumptively identified as VRE pending confirmatory tests.[1]

References

1. Barton and Doern. 1995. Diagn. Microbiol. Infect. Dis. *23*:119.
2. Jett, Huycke and Gilmore. 1994. Clin. Microbiol. Rev. *7*:462.
3. Moellering. 1992. Clin. Infect. Dis. *14*:1173.
4. Emori and Gaynes. 1993. Clin. Microbiol. Rev. *6*:428.
5. Landry, Kaiser and Wenzel. 1989. Am. J. Infect. Control. *17*:323.
6. Subcommittee on Prevention and Control of Antimicrobial-Resistant Microorganisms in Hospitals. 1994. Preventing the spread of vancomycin resistance: a report from the hospital infection control practices advisory committee. Fed. Regist. *59*:25758.
7. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott, Williams & Wilkins, Baltimore, Md.
8. Clinical and Laboratory Standards Institute. 2006. Approved standard: M2-A9. Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.

Availability

BBL™ Enterococcosel™ Agar with Vancomycin, 8 µg/mL

BS12 CLSI

Cat. No. 292234 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

E

m Enterococcus Agar

Intended Use

m Enterococcus Agar, also referred to as m Azide Agar, is used for isolating and enumerating enterococci in water and other materials by membrane filtration or pour plate technique.

Summary and Explanation

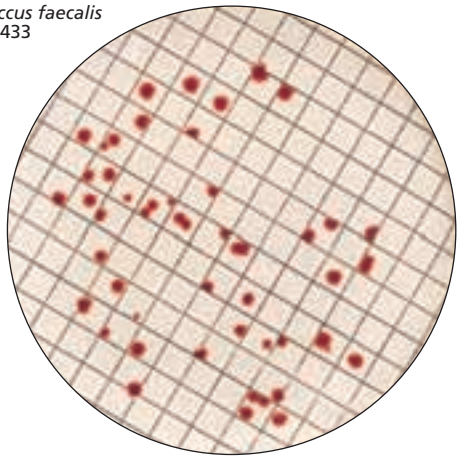
The enterococcus group is a subgroup of the fecal streptococci that includes *E. faecalis, E. faecium, E. gallinarum,* and *E. avium*.[1] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C.[1] The enterococcal portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1] m Enterococcus Agar is used in standard methods for the detection of fecal streptococcus and enterococcus groups using the membrane filtration technique.[1]

m Enterococcus Agar was developed by Slanetz et al.[2] for the enumeration of enterococci by the membrane filtration technique. A modification of m Enterococcus Agar, adding triphenyltetrazolium chloride (TTC), was described by Slanetz and Bartley[3]. This modified medium proved to be a superior membrane filtration medium for the enumeration of enterococci. Increased recovery and larger colonies were obtained by incubating the inoculated membranes on the agar surface instead of on pads saturated with liquid medium. The membrane filtration method has the advantages of being simpler to perform, not requiring confirmation and permitting a direct count of enterococci in 48 hours. Burkwell and Hartman[4] added 0.2% sodium carbonate and 0.05% polysorbate 80 to m Enterococcus Agar to increase the sensitivity for the direct plating method.

Principles of the Procedure

Peptone provides nitrogen, minerals and amino acids. Yeast extract is the vitamin source and dextrose supplies carbon. Dipotassium phosphate acts as a buffer for the medium.

Enterococcus faecalis
ATCC™ 19433



User Quality Control

Identity Specifications

Difco™ m Enterococcus Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 4.2% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ m Enterococcus Agar

Prepare the medium per label directions. Inoculate using the membrane filter technique. Incubate in humid atmosphere at 35 ± 0.5°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	19433	20-60	Good	Light pink to red
Enterococcus faecalis	29212	20-60	Good	Light pink to red
Escherichia coli	25922	10^3	Marked to complete inhibition	–

Sodium azide is the selective agent to suppress the growth of gram-negative organisms. Agar is the solidifying agent. Triphenyl tetrazolium chloride (TTC) is the dye used as an indicator of bacterial growth. TTC is reduced to the insoluble formazan inside the bacterial cell, resulting in the production of red colonies.

Formula

Difco™ m Enterococcus Agar

Approximate Formula* Per Liter

Tryptose	20.0 g
Yeast Extract	5.0 g
Dextrose	2.0 g
Dipotassium Phosphate	4.0 g
Sodium Azide	0.4 g
Agar	10.0 g
2,3,5-Triphenyl Tetrazolium Chloride	0.1 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 42 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and dispense into 50 x 9 mm Petri dishes to a depth of 4-5 mm (approximately 4-6 mL).
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Collect water samples as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9060[1] or by laboratory policy.

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9230C.[1]
2. Choose a sample size so that 20-60 colonies will result.
3. Transfer the filter to agar medium in a Petri dish, avoiding air bubbles beneath the membrane.
4. Let plates stand for 30 minutes.
5. Invert plates and incubate at 35 ± 0.5°C for 48 hours.

Direct plating procedure

1. Inoculate medium with a specimen using the streak plate method.
2. Incubate plates at 35 ± 2°C for 24-48 hours.

Expected Results[1]

Count all light and dark red colonies as enterococci. Count colonies using a fluorescent lamp and a magnifying lens.

References

1. Eaton, Rice and Baird (ed). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Slanetz, Bent and Bartley. 1955. Public Health Rep. *70*:67.
3. Slanetz and Bartley. 1957. J. Bacteriol. *74*:591.
4. Burkwell and Hartman. 1964. Appl. Microbiol. *12*:18.

Availability

Difco™ m Enterococcus Agar

ISO SMWW

Cat. No.	274610	Dehydrated – 100 g
	274620	Dehydrated – 500 g

Enterococcus Screen Agar QUAD Plate with Streptomycin/Gentamicin/Vancomycin

Intended Use

Enterococcus Screen Agar is used to test enterococci for high-level resistance to aminoglycosides and vancomycin to predict the synergistic activity of these antimicrobials.

Summary and Explanation

Enterococci are known to cause a wide variety of infections. Most commonly they infect the urinary tract, abdomen, bloodstream, endocardium, biliary tract, burn wounds and in-dwelling catheters.[1] *Enterococcus faecalis* causes 80 to 90% of infections, while *E. faecium* causes the majority of the remainder.[2] Today

the enterococci are the fourth leading cause of bacteremia in the United States.[3] The case/fatality rates for enterococcal bacteremia range from 12 to 68% with death due to sepsis in 4 to 50% of the cases.[4]

Treatment of enterococcal infections with either penicillin or vancomycin alone fails to kill enterococci resulting in relapse of infection.[5] Enterococci for years were known to have low intrinsic resistance to a variety of β-lactam as well as aminoglycoside antibiotics.[6] The addition of an aminoglycoside to which the isolate has demonstrated susceptibility results in both *in vitro* and *in vivo* synergism producing a bactericidal effect.[7] This synergistic effect is thought to be due to the penicillin or vancomycin damaging the integrity of the cell wall, thus allowing the aminoglycoside to penetrate and inhibit bacterial protein synthesis.[8] The emergence of high level resistance to streptomycin (≥ 2000 µg/mL), gentamicin (≥ 500 µg/mL) and vancomycin (≥ 6 µg/mL) results in the failure of the penicillin- or vancomycin-aminoglycoside combinations to eradicate the infecting organisms. Therefore, testing for high level resistance to streptomycin, gentamicin and vancomycin is important. The use of a Brain Heart Infusion Agar (BHIA) containing streptomycin (2000 µg/mL), gentamicin (500 µg/mL) or vancomycin (6 µg/mL) is recommended by the Clinical and Laboratory Standards Institute (CLSI) for testing high level resistance.[9]

Principles of the Procedure

Brain Heart Infusion Agar is a general-purpose medium suitable for the cultivation of a wide variety of microorganisms and is recommended for agar screen susceptibility testing of enterococci.[9]

The meat infusion solids and peptones are sources of organic nitrogen, carbon, sulfur, vitamins, and trace substances. Dextrose is the carbohydrate source. The medium is buffered through the use of disodium phosphate. Streptomycin at 2000 µg/mL and gentamicin at 500 µg/mL are used to detect high level aminoglycoside resistance.[9] Vancomycin at 6 µg/mL is used to detect resistance to vancomycin.[9] The Food, Drug & Cosmetic (FD&C) dyes are inert and added for easy visual identification of the antimicrobials.

Enterococcus faecalis
ATCC™ 51299

Procedure

1. Prepare the inoculum by suspending several well-isolated colonies of the enterococcal isolate from an 18-24 hour plate culture into a tube of **Trypticase**™ Soy Broth and adjust the turbidity to be equivalent to a 0.5 McFarland turbidity standard.
2. Spot inoculate each quadrant of the plate with 10 µL of the adjusted suspension.
3. Allow the inoculum spots to absorb into the agar surfaces.
4. Incubate plates at 35 ± 2°C aerobically for a full 24 hours. If negative at 24 hours, reincubate streptomycin tests an additional 24 hours.

Expected Results

Following a full 24 hours of incubation, observe plates for growth. Growth on Quadrant I (BHIA Control) indicates viable test organisms in the inoculum broth suspension and the test is valid. If there is no growth, the test is invalid and must be repeated.

Growth on:

Quadrant II – Red (BHIA with gentamicin) and/or
Quadrant III – Yellow (BHIA with vancomycin) and/or
Quadrant IV – Blue (BHIA with streptomycin) indicates that the antimicrobial would not be synergistic in combination therapy.

No growth indicates synergy may be predicted. See the CLSI standard and supplemental tables for details of interpretation and additional procedures.

References

1. Jett, Huycke and Gilmore. 1994. Clin. Microbiol. Rev. 7:462.
2. Moellering. 1992. Clin. Infect. Dis. *14*:1173.
3. Emori and Gaynes. 1993. Clin. Microbiol. Rev. *6*:428.
4. Landry, Kaiser and Wenzel. 1989. Am. J. Infect. Control *17*:323.
5. Moellering, Korzeniowski, Sande and Wennersten. 1979. J. Infect. Dis. *140*:203.
6. Murray. 1990. Clin. Microbiol. Rev. *3*:46.
7. Mandell. 1984. Ann. Intern. Med. *100*:904.
8. Moellering and Weinberg. 1971. J. Clin. Invest. *50*:2580.
9. Clinical and Laboratory Standards Institute. 2006. Approved standard: M7-A7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. CLSI, Wayne, Pa.

Availability

BBL™ Enterococcus Screen Agar QUAD Plate with Streptomycin/Gentamicin/Vancomycin

BS12 CLSI CMPH2

Cat. No. 222201 Prepared Plates (QUAD) – Pkg. of 10*

**Store at 2-8°C.*

Eosin Methylene Blue Agar, Levine
EMB Agar, Levine, without Lactose

Intended Use

Eosin Methylene Blue Agar, Levine is a slightly selective and differential plating medium for the isolation of gram-negative enteric bacteria. EMB Agar, Levine, without Lactose is provided for convenience in genetic studies of enteric bacilli.

Summary and Explanation

Shortly following the publication by Holt-Harris and Teague of a paper describing a new culture medium for the differentiation of enteric microorganisms through the use of eosin and methylene blue dyes,[1] Levine described a modification of their formulation which he claimed gave better differentiation between what are now referred to as *Escherichia* and *Enterobacter* species.[2] The two formulations differ in that Levine EMB Agar does not contain sucrose. Both of these formulations were developed to improve upon the differentiating properties of Endo Agar,[3] which was developed previously.

Levine EMB Agar has become the predominant enteric plating medium that utilizes dyes as selective agents. It is recommended for use in the microbiological examination of dairy products and foods by the American Public Health Association.[4,5] EMB Agar, Levine, without Lactose can be supplemented with a carbohydrate as the sole carbon source for the study of hybrid enteric bacilli.[6]

Principles of the Procedure

The eosin Y and methylene blue dyes in Levine EMB Agar render the medium slightly selective in that they inhibit gram-

User Quality Control

Identity Specifications

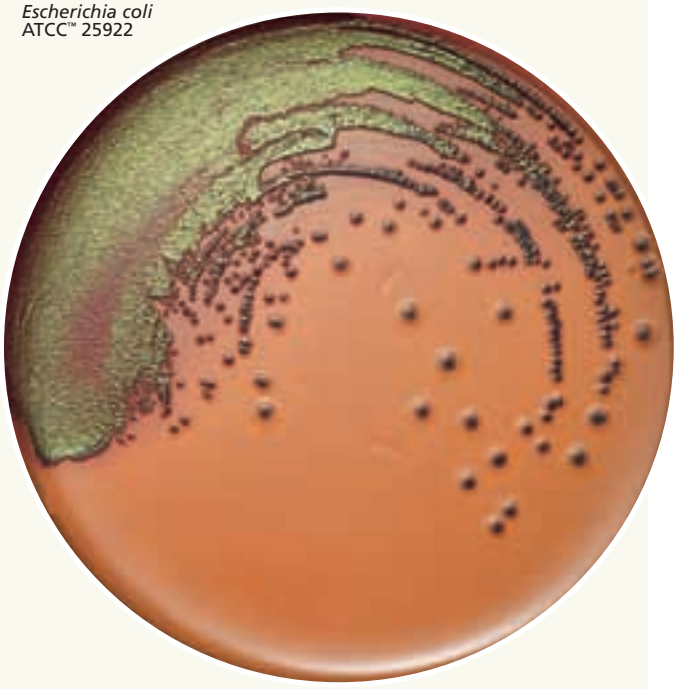
BBL™ Eosin Methylene Blue Agar, Levine

Dehydrated Appearance:	Fine, homogeneous, may contain up to a large amount of minute to small dark red purple particles.
Solution:	3.74% solution, soluble in purified water upon boiling. Solution is medium to dark, green orange brown, hazy.
Prepared Appearance:	Medium to dark, green orange brown, hazy.
Reaction of 3.74% Solution at 25°C:	pH 7.1 ± 0.2

BBL™ EMB Agar, Levine, without Lactose

Dehydrated Appearance:	Fine, homogeneous, may contain up to a large amount of minute to small dark red purple particles.
Solution:	2.74% solution, soluble in purified water upon boiling. Solution is medium to dark, green orange brown, hazy.
Prepared Appearance:	Medium to dark, green orange brown, hazy.
Reaction of 2.74% Solution at 25°C:	pH 7.1 ± 0.2

Escherichia coli
ATCC™ 25922



Cultural Response

BBL™ Eosin Methylene Blue Agar, Levine or EMB Agar, Levine, without Lactose

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	10^4-10^5	Partial inhibition
Escherichia coli	25922	10^3-10^4	Good
Klebsiella pneumoniae	33495	10^3-10^4	Good
Salmonella enterica subsp. *enterica* serotype Typhi	19430	10^3-10^4	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good
Shigella dysenteriae	9361	10^3-10^4	Good
Shigella flexneri	12022	10^3-10^4	Good

positive bacteria to a limited degree. These dyes also play a role in differentiating between lactose fermenters and lactose nonfermenters due to the presence or absence of dye uptake in the bacterial colonies. Coliforms, as lactose-fermenting organisms, are visualized as blue-black colonies, whereas colonies of *Salmonella* and *Shigella*, as lactose nonfermenters, appear colorless, transparent or amber.

Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts, will grow on this medium and usually form pinpoint colonies. A number of nonpathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic strains by additional biochemical tests.

Formulae

BBL™ Eosin Methylene Blue Agar, Levine

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	10.0	g
Lactose	10.0	g
Dipotassium Phosphate	2.0	g
Eosin Y	0.4	g
Methylene Blue	65.0	mg
Agar	15.0	g

BBL™ EMB Agar, Levine, without Lactose

Consists of the same ingredients without the lactose.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   BBL™ Eosin Methylene Blue Agar, Levine – 37.4 g;
   BBL™ EMB Agar, Levine, without Lactose – 27.4 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Follow established procedures when using the medium without lactose.

Expected Results

Typical colonial morphology on Eosin Methylene Blue Agar, Levine is as follows:

Escherichia coli	Large, blue-black, green metallic sheen
Enterobacter/Klebsiella	Large, mucoid, blue-black
Proteus	Large, colorless
Salmonella	Large, colorless
Shigella	Large, colorless
Pseudomonas	Irregular, colorless
Gram-positive bacteria	No growth to slight growth

Results obtained with Levine EMB Agar without Lactose are dependent upon the substituted carbohydrate.

References

1. Holt-Harris and Teague. 1916. J. Infect. Dis. *18*:596.
2. Levine. 1918. J. Infect. Dis. *23*:43.
3. Endo. 1904. Zentralbl. Bakteriol., Abt. 1, Orig. *35*:109.
4. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Baron, Spilman and Carey. 1959. Abstr. G7, p. 29. Bacteriol. Proc. 59th Gen. Meet. Soc. Am. Bacteriologists 1959.

Availability

BBL™ Eosin Methylene Blue Agar, Levine

AOAC | BAM | BS12 | CCAM | CMPH2 | COMPF | MCM9 | SMD

Cat. No.	211221	Dehydrated – 500 g
	211222	Dehydrated – 5 lb (2.3 kg)
	221170	Prepared Plates – Pkg. of 20*
	221268	Prepared Plates – Ctn. of 100*

BBL™ EMB, Levine, without Lactose

Cat. No. 211191 Dehydrated – 500 g

BBL™ Eosin Methylene Blue Agar, Levine// Columbia CNA Agar with 5% Sheep Blood

Cat. No. 295618 Prepared **I Plate™** Dishes – Ctn. of 100*

BBL™ Eosin Methylene Blue Agar, Levine// MacConkey II Agar

Cat. No. 295969 Prepared **I Plate™** Dishes – Ctn. of 100*

BBL™ Eosin Methylene Blue Agar, Levine// Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No. 221286 Prepared **I Plate™** Dishes – Pkg. of 20*

**Store at 2-8°C.*

Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague

Intended Use

Eosin Methylene Blue Agar, Modified (formula of Holt-Harris and Teague) is a slightly selective and differential medium for the isolation, cultivation and differentiation of gram-negative enteric bacilli from both clinical and nonclinical specimens.

Summary and Explanation

In 1904, Endo developed a culture medium for the isolation of typhoid bacilli from feces,[1] and this medium was widely used in the years immediately following its development. According to Holt-Harris and Teague,[2] the chief disadvantage of the Endo medium was that the red color of the coliform colonies diffused through the surrounding medium. When larger numbers of these colonies were present on the agar surface, the colorless colonies of the typhoid organisms and other lactose nonfermenters were masked and often overlooked. In 1916, these two scientists reported on the development of a new medium in which the dyes, eosin Y and methylene blue, were incorporated. Differentiation between lactose fermenters and lactose nonfermenters on this formulation was greatly improved since color diffusion into the agar was eliminated.

The original EMB Agar formulation of Holt-Harris and Teague was modified by Levine who described his medium in a 1918 publication.[3] Levine simplified the original formula by using a single peptone as a base and supplementing it with dipotassium phosphate as a buffer and by deleting the sucrose and increasing the concentration of lactose. The concentration of methylene blue was later reduced because of increased purity of the dye. This provided the current ratio of eosin to methylene blue of approximately 6:1. Over the years, it is the Levine Eosin Methylene Blue formulation that has achieved dominant status.

Principles of the Procedure

Eosin Methylene Blue Agar, Modified, contains eosin Y and methylene blue dyes that inhibit gram-positive bacteria to a limited degree. The dyes also serve as differential indicators in response to the fermentation of lactose and/or sucrose by microorganisms. Coliforms produce blue-black colonies due to the taking up of an eosin-methylene blue dye complex by the bacterial cells when the pH drops. *Salmonella* and *Shigella* colonies are colorless or have a transparent amber color. *Escherichia coli* colonies may show a characteristic green metallic sheen due to the rapid fermentation of lactose.

Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts, will grow on this medium and usually form pinpoint colonies. A number of non-pathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.

User Quality Control

Identity Specifications

BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague

Dehydrated Appearance:	Fine, homogeneous, may contain up to a large amount of minute to small dark red purple particles.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is medium to dark, green orange brown, hazy.
Prepared Appearance:	Medium to dark, green orange brown, hazy.
Reaction of 3.6% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	10^4-10^5	Partial inhibition
Escherichia coli	25922	10^3-10^4	Good
Proteus vulgaris	9484	10^3-10^4	Good
Salmonella enterica subsp. *enterica* serotype Typhi	19430	10^3-10^4	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good
Shigella flexneri	12022	10^3-10^4	Good

Formula

BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	10.0	g
Lactose	5.0	g
Sucrose	5.0	g
Dipotassium Phosphate	2.0	g
Eosin Y	0.4	g
Methylene Blue	65.0	mg
Agar	13.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to approximately 45°C. Agitate gently and pour into plates.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Use standard procedures to obtain isolated colonies from specimens. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Expected Results
Typical colonial morphology on EMB Agar, Modified is as follows:

Escherichia coli	Large, blue-black, green metallic sheen
Enterobacter/Klebsiella	Large, mucoid, blue-black
Proteus	Large, colorless
Salmonella	Large, colorless to amber
Shigella	Large, colorless to amber
Pseudomonas	Irregular, colorless
Gram-positive bacteria	No growth to slight growth

References

1. Endo. 1904. Zentralbl. Bakteriol., Abt. I Orig. *35*:109.
2. Holt-Harris and Teague. 1916. J. Infect. Dis. *18*:596.
3. Levine. 1918. J. Inf. Dis. *23*:43.

Availability
BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague
AOAC

Cat. No. 211215 Dehydrated – 500 g

United States and Canada

Cat. No. 221354 Prepared Plates – Pkg. of 20*
221355 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254014 Prepared Plates – Pkg. of 20*
254073 Prepared Plates – Ctn. of 120*

BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague//Columbia CNA Agar with 5% Sheep Blood
Cat. No. 221941 Prepared **I Plate**™ Dishes – Pkg. of 20*

**Store at 2-8°C.*

Esculin Agar

Intended Use
Esculin Agar is a differential medium for demonstrating esculin hydrolysis by various microorganisms.

Summary and Explanation
Esculin hydrolysis is recommended in the differentiation and identification of a variety of organisms.[1-3] If the test organism does not hydrolyze esculin, the medium remains unchanged and the esculin will fluoresce when subjected to long-wave UV light at 360 nm. When hydrolyzed, the medium turns black and fluorescence is lost.[1]

Principles of the Procedure
Animal tissue peptones and infusions from heart muscle provide amino acids or other nitrogenous substances that support bacterial growth. Sodium chloride maintains osmotic equilibrium.

Esculin is a glycoside incorporated as a differential agent to facilitate the identification of various organisms, including *Enterobacteriaceae*, enterococci and anaerobes. Hydrolysis of esculin yields esculetin and dextrose. In the presence of an iron salt, esculetin forms a brown-black complex that diffuses into the surrounding medium.[3]

Procedure
Organisms to be tested must first be isolated in pure culture on an appropriate solid medium. Using a sterile inoculating loop or needle, inoculate esculin agar with several isolated colonies. Incubate tubes at 35°C with caps loosened for up to 48 hours.

Expected Results
Blackening of the agar medium in the area of growth indicates esculin hydrolysis.

References

1. Shigei. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
2. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.

Availability
BBL™ Esculin Agar
Cat. No. 295951 Prepared Slants – Pkg. of 10*

**Store at 2-8°C.*

Esculin Iron Agar
(See m E Agar)

Eugon Agar

Intended Use

Eugon Agar is a general-purpose medium used for cultivating a wide variety of microorganisms.

Summary and Explanation

Eugon Agar is prepared according to the formula described by Pelczar and Vera.[1] Eugon Agar and Eugon Broth were developed to obtain eugonic (luxuriant) growth of fastidious microorganisms.[2] Eugon Agar can be used with or without enrichment. Enriched with blood, Eugon Agar supports the growth of pathogenic fungi including *Nocardia*, *Histoplasma* and *Blastomyces*. With the addition of Supplement B, excellent growth of *Neisseria*, *Francisella* and *Brucella* is achieved. The unenriched medium supports rapid growth of lactobacilli associated with cured meat products, dairy products and other foods.

Niven[3] reported the use of Eugon Agar for the detection of lactic acid in cured meats, and recommended it for investigating spoilage in meats. Harrison and Hansen[4] employed the medium for plate counts of the intestinal flora of turkeys. Frank[5] showed its usefulness in germinating anaerobic spores pasteurized at 104°C.

Eugon Agar is included in the *Compendium of Methods for the Microbiological Examination of Foods*.[6]

User Quality Control

Identity Specifications

Difco™ Eugon Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.54% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent, cystine precipitate may be visible.
Prepared Appearance:	Light amber, slightly opalescent, cystine precipitate may be visible.
Reaction of 4.54% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Eugon Agar

Prepare the medium (unsupplemented) per label directions. For *Candida albicans* and *Aspergillus brasiliensis* inoculate using fresh broth cultures and incubate at 30 ± 2°C for 18-48 hours. For all other cultures inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	Fresh	Fair to good
Candida albicans	26790	Fresh	Good
Lactobacillus fermentum	9338	30-300	Good
Shigella flexneri	12022	30-300	Good
Streptococcus pyogenes	19615	30-300	Good

Principles of the Procedure

Peptones provide the nitrogen, vitamins and amino acids in Eugon Agar. The high concentration of dextrose is the energy source for rapid growth of bacteria. L-Cystine and sodium sulfite are added to stimulate growth. Sodium chloride maintains the osmotic balance of the media. The high carbohydrate content along with high sulfur (cystine) content improves growth with chromogenicity.[2] Agar is the solidifying agent in Eugon Agar.

Formula

Difco™ Eugon Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	7.5	g
Pancreatic Digest of Casein	7.5	g
Soy Peptone	5.0	g
Dextrose	5.5	g
L-Cystine	0.7	g
Sodium Chloride	4.0	g
Sodium Sulfite	0.2	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45.4 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. When an enrichment is being prepared, cool to 50-55°C prior to adding the desired enrichment.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on bacteria and fungi from clinical specimens, refer to the appropriate procedures outlined in the references.[7,8] For the examination of bacteria and fungi in food refer to standard methods.[6,9]

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Eugon Agar is not recommended as a blood agar base for hemolytic reactions because of its high sugar content.
2. It is suggested that Eugon Agar be prepared as required. Do not melt and resolidify media containing enrichments.

References

1. Pelczar and Vera. 1949. Milk Plant Monthly *38*:30.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Niven. 1949. J. Bacteriol. *58*:633.
4. Harrison and Hansen. 1950. J. Bacteriol. *59*:197.
5. Frank. 1955. J. Bacteriol. *70*:269.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
9. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Eugon Agar

COMPF

Cat. No. 258910 Dehydrated – 500 g

Difco™ Supplement B

Cat. No. 227610 Lyophilized – 6 × 10 mL with Reconstituting Fluid*
227620 Lyophilized – 100 mL with Reconstituting Fluid*

**Store at 2-8°C.*

Bacto™ Eugon Broth • Eugonbroth™ Medium

Intended Use

Eugon Broth (**Eugonbroth**™) is a general-purpose medium used for the cultivation of fastidious and nonfastidious bacteria from a variety of clinical and nonclinical specimens.

Summary and Explanation

Eugon Broth (**Eugonbroth**) is the fluid form of Eugon Agar, a clear medium developed for use in the enumeration of bacteria in milk and other products.[1] The formulation was developed from a study conducted by Vera of various peptones, carbohydrates, salts and other constituents in various concentrations and combinations to yield eugonic (luxuriant) growth of bacteria.[2]

Principles of the Procedure

Peptones supply amino acids and other nitrogenous substances to support bacterial growth. L-cystine is an essential amino acid that improves growth. Dextrose is incorporated as a source of energy and sodium chloride provides osmotic equilibrium. Sodium sulfite along with the cystine content improves growth with chromogenicity.

Formula

Bacto™ Eugon Broth

Approximate Formula* Per Liter		
Proteose Peptone No. 3	7.5	g
Pancreatic Digest of Casein	7.5	g
Soy Peptone	5.0	g
Dextrose	5.5	g
L-Cystine	0.7	g
Sodium Chloride	4.0	g
Sodium Sulfite	0.2	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 30.4 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. When an enriched medium is being prepared, cool to 50-55°C prior to adding the desired enrichment.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Bacto™ Eugon Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.04% solution, soluble in purified water upon boiling. Solution is light amber, clear, may contain up to a large amount of precipitate.
Prepared Appearance:	Light amber, clear, may have a slight precipitate.
Reaction of 3.04% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Bacto™ Eugon Broth

Prepare the medium (unsupplemented) per label directions. Inoculate and incubate with caps loosened at 35 ± 2°C (*Aspergillus brasiliensis* and *Candida albicans* at 30 ± 2°C) for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Fair to good
Candida albicans	26790	30-300	Good
Lactobacillus fermentum	9338	30-300	Good
Shigella flexneri	12022	30-300	Good
Streptococcus pyogenes	19615	30-300	Good

Procedure

Organisms to be cultivated must first be isolated in pure culture on an appropriate solid medium.

Using a sterile inoculating loop or needle, transfer fresh growth from the subculture medium to the tubed medium.

Incubate under conditions appropriate for the organism being cultivated. Broth cultures should be held at least 1 week before discarding as negative.

Expected Results

Growth in tubes is indicated by the presence of turbidity compared to an uninoculated control.

If growth appears, cultures should be examined by Gram staining, subculturing onto appropriate media and incubating inoculated media aerobically with increased CO_2 and/or anaerobically.

References

1. Pelczar and Vera. 1949. Milk Plant Monthly. *38*:30.
2. Vera. 1947. J. Bacteriol. *54*:14.

Availability

Bacto™ Eugon Broth

Cat. No. 259010 Dehydrated – 500 g

BBL™ Eugonbroth™ Medium

Cat. No. 297424 Prepared Tubes – Ctn. of 100*

**Store at 2-8°C.*

m FC Agar • m FC Broth Base Rosolic Acid

Intended Use

m FC Agar and m FC Broth Base are used with Rosolic Acid in cultivating and enumerating fecal coliforms by the membrane filter technique at elevated temperatures.

Summary and Explanation

Geldreich et al.[1] formulated a medium to enumerate fecal coliforms (MFC) using the membrane filter (MF) technique without prior enrichment. Fecal coliforms (i.e., those found in the feces of warm-blooded animals) are differentiated from coliforms from environmental sources by their ability to grow at 44.5 ± 0.5°C.[2]

Many "standard methods" membrane filtration procedures specify m FC medium for testing water.[2-4] The American Public Health Association (APHA) specifies m FC medium and incubation at 44.5 ± 0.5°C in the fecal coliform membrane filter procedure, the delayed-incubation fecal coliform procedure and the two-layer agar method for recovering injured fecal coliforms.[2] AOAC International specifies m FC Agar for detecting total coliforms and fecal coliforms in foods.[3]

The U. S. Environmental Protection Agency specifies using m FC medium in fecal coliform methods for testing water by the direct MF method or the delayed-incubation MF method.[4,5]

Principles of the Procedure

m FC Agar and m FC Broth Base contain peptones as sources of carbon, nitrogen, vitamins and minerals.Yeast extract supplies B-complex vitamins that stimulate bacterial growth. Lactose is a carbohydrate. Bile Salts No. 3 inhibits growth of gram-positive bacteria. m FC Agar contains agar as the solidifying agent. The differential indicator system combines aniline blue and rosolic acid.

Colonies of fecal coliforms are blue; non-fecal coliforms and other organisms are gray to cream-colored.

Formulae

Difco™ m FC Agar

Approximate Formula* Per Liter

Tryptose	10.0	g
Proteose Peptone No. 3	5.0	g
Yeast Extract	3.0	g
Lactose	12.5	g
Bile Salts No. 3	1.5	g
Sodium Chloride	5.0	g
Agar	15.0	g
Aniline Blue	0.1	g

Difco™ m FC Broth Base

Consists of the same ingredients without the agar.

Difco™ Rosolic Acid

Rosolic Acid	1	g/vial

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ m FC Agar

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 10 mL of a 1% solution of Rosolic Acid in 0.2N NaOH. Continue heating for 1 minute. DO NOT AUTOCLAVE.
4. If necessary, adjust to pH 7.4 with 1N HCl.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ m FC Broth Base

1. Suspend 3.7 g of the powder in 100 mL of purified water.
2. Add 1 mL of a 1% solution of Rosolic Acid in 0.2N NaOH.
3. If necessary, adjust to pH 7.4 with 1N HCl.
4. Heat to boiling. DO NOT AUTOCLAVE.
5. Cool before dispensing.
6. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Rosolic Acid

Prepare a 1% solution, dissolving 1 g in 100 mL of 0.2N NaOH.

User Quality Control

Identity Specifications

Difco™ m FC Agar

Dehydrated Appearance:	Beige with a slight blue tint to blue, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Without 1% Rosolic Acid: blue, very slightly to slightly opalescent, may have a slight precipitate. With 1% Rosolic Acid: cranberry red, slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Without 1% Rosolic Acid—Blue, slightly opalescent, may have slight precipitate. With 1% Rosolic Acid—Cranberry red, slightly opalescent, may have slight precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2 (without 1% Rosolic Acid)

Difco™ m FC Broth Base

Dehydrated Appearance:	Beige with a slight blue tint to blue, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is blue, slightly opalescent, may have a precipitate.
Prepared Appearance:	Without 1% Rosolic Acid—Blue, slightly opalescent, may have a very fine precipitate. With 1% Rosolic Acid—Cranberry red, slightly opalescent, may have a slight precipitate.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2 (without 1% Rosolic Acid)

Difco™ Rosolic Acid

Dehydrated Appearance:	Dark reddish-brown with metallic green particles, free-flowing, fine crystalline powder.
Solution:	1.0% solution, soluble in 0.2N NaOH. Solution is deep red, clear to very slightly opalescent.

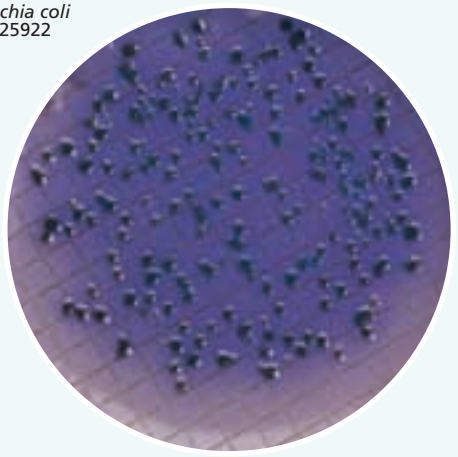
Cultural Response

Difco™ m FC Agar or m FC Broth Base

Prepare the medium per label directions with 1% Rosolic Acid. Using the membrane filter technique, inoculate and incubate plates at 44.5 ± 0.5°C for 24 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	19433	10^3-2×10^3	Marked to complete inhibition	–
Escherichia coli	25922	20-80	Good	Blue

Escherichia coli ATCC™ 25922



F
G

Procedure

Difco™ m FC Agar

1. Prepare the agar medium from the dehydrated base according to the label directions and with the addition of the Rosolic Acid solution.
2. Pour molten agar, previously cooled to 45-50°C into special tight-fitting plastic dishes and allow to harden.
3. Roll the membrane filter used to collect the water sample onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
4. Place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

Difco™ m FC Broth

1. Prepare the broth medium from the dehydrated base according to the label directions and with the addition of the Rosolic Acid solution.
2. Add 2 mL of the cooled broth to sterile absorbent pads in special tight-fitting plastic dishes.
3. Roll the membrane filter used to collect the water sample onto the moistened absorbent pad, so as to avoid the formation of air bubbles between the filter and the pad.
4. Place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

Expected Results

Colonies of fecal coliforms will be various shades of blue. Non-fecal coliforms are gray to cream-colored.

Limitation of the Procedure

A few non-fecal coliform colonies may be observed on m FC media due to the selective action of the elevated temperature and the addition of the Rosolic Acid. It may be useful to elevate the temperature to 45 ± 0.2°C to eliminate *Klebsiella* strains from the fecal coliform group.[6]

References

1. Geldreich, Huff and Best. 1965. J. Am. Water Works Assoc. 57:208.
2. Eaton, Rice and Baird (ed). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International. Gaithersburg, Md.
4. U.S. Environmental Protection Agency. 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, USEPA, Cincinnati, Ohio.
5. Bordner, Winter and Scarpino (ed.). 1978. Microbiological methods for monitoring the environment: water and wastes. Publication EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.
6. Eaton, Clesceri and Greenberg (ed.). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ m FC Agar

AOAC CCAM EPA SMWW

Cat. No. 267710 Dehydrated – 100 g
267720 Dehydrated – 500 g

Difco™ m FC Broth Base

EPA SMWW

Cat. No. 288320 Dehydrated – 100 g
288330 Dehydrated – 500 g

Difco™ Rosolic Acid

Cat. No. 232281 Vial – 6 × 1 g

m FC Basal Medium

Intended Use

m FC Basal Medium is used with MUG or BCIG for cultivating and enumerating fecal coliforms by the membrane filter technique at elevated temperatures.

Summary and Explanation

Ciebin et al.[1] described a modification of m FC Medium, called FC Basal Medium, in which the chromogenic substrate 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (BCIG) is added for quantitative recovery of *Escherichia coli* from untreated water samples to show fecal contamination using membrane filter methods.

Standard method procedures use media with the fluorogenic substrate, 4-methylumbelliferyl-β-D-glucuronide (MUG) to enumerate *E. coli* by membrane filter methods.[2] Disadvantages of using MUG include the requirement of ultraviolet light, possible diffusion of fluorescence from the colony to the surrounding medium and background fluorescence of membrane filters.[3] Using BCIG in place of MUG to detect β-glucuronidase activity gives visible blue colonies and an indigo-blue complex that remains within the colony. Ciebin et al.[1] found FC-BCIG Medium comparable to standard MUG-based media for detection of β-glucuronidase activity of *E. coli*.

In another study, Ciebin et al.[4] formulated DC Medium using FC Basal Medium supplemented with lactose, BCIG and cefsulodin. It is a differential coliform medium for the enumeration of coliforms and *E. coli* in potable water using membrane filtration. Ciebin et al. compared DC Medium to LES Endo Medium and FC-BCIG Medium. They found DC Medium superior to LES Endo Medium in recovering coliforms and equivalent to FC-BCIG Medium in recovering *E. coli*.

Principles of the Procedure

m FC Basal Medium contains peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins that stimulate bacterial growth. Bile Salts No. 3 inhibits the growth of gram-positive microorganisms. Agar is the solidifying agent.

Formula

Difco™ m FC Basal Medium

Approximate Formula* Per Liter		
Tryptose	10.0	g
Proteose Peptone No. 3	5.0	g
Yeast Extract	3.0	g
Bile Salts No. 3	1.5	g
Sodium Chloride	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ m FC Basal Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent, may have slight precipitate.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

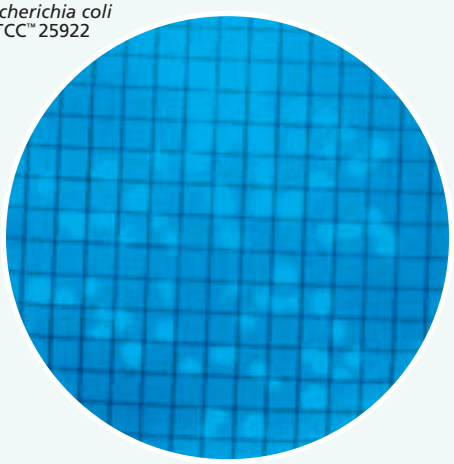
Cultural Response

Difco™ m FC Basal Medium

Prepare the medium per label directions (with the addition of 0.01% MUG). Using the membrane filter technique, inoculate and incubate at 44.5 ± 0.5°C for 24 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	FLUORESCENCE
Enterobacter aerogenes	13048	30-200	Inhibition	–
Enterococcus faecalis	19433	3×10^{2}-10^{3}	Marked to complete inhibition	–
Escherichia coli	25922	30-200	Good	+ (blue-white)

Escherichia coli ATCC™ 25922



Directions for Preparation from Dehydrated Product

1. Suspend 39.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Add 100 mg of MUG or BCIG, as desired, and boil to completely dissolve the powder. DO NOT AUTOCLAVE.
3. If necessary, adjust to pH 7.4 with 1N HCl.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Filter duplicate water samples through membrane filtration apparatus.
2. Transfer each membrane to the surface of the m FC Basal Medium plate. Place cultures in waterproof plastic bags. Submerge Petri dishes in waterbath. Incubate one set of plates at 35 ± 2°C and one set at 44.5 ± 0.2°C for 24 ± 2 hours.
3. Count number of colonies and record observations. m FC-MUG plates are read under long-wavelength (366 nm) ultraviolet light.

Expected Results

m FC-MUG

β-glucuronidase-positive organisms produce a blue-white fluorescence; e.g., *E. coli*.

β-glucuronidase-negative organisms do not fluoresce; e.g., *E. aerogenes, E. faecalis, S.* Typhimurium, *S. flexneri*.

m FC-BCIG

β-glucuronidase-positive organisms produce a visible blue colony.

β-glucuronidase-negative organisms produce a non-blue colony.

References

1. Ciebin, Brodsky, Eddington, Horsnell, Choney, Palmateer, Ley, Joshi and Shears. 1995. Appl. Environ. Microbiol. *61*:3940.
2. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
3. Farber. 1986. Can. Inst. Food Sci. Tech. J. *19*:34.
4. Ciebin, Schop and Brodsky. 1997. Q-152. Abstr. 97th Annu. Meet. Am. Soc. Microbiol. 1997.

Availability

Difco™ m FC Basal Medium

Cat. No. 269810 Dehydrated – 500 g

FLN Agar

Intended Use

FLN (Fluorescence Lactose Nitrate) Agar was developed to provide a screening procedure for partial identification of nonfermentative gram-negative bacilli.

Summary and Explanation

Pickett and Pedersen observed that over 50% of 183 strains of nonfermentative bacilli from clinical specimens consisted of only 3 (of 17) species.[1] These species were *Pseudomonas aeruginosa*, *Pseudomonas maltophilia* (now *Stenotrophomonas maltophilia*), and *Achromobacter anitratus* (now *Acinetobacter baumannii*). This observation led to the development of media and tests to differentiate and partially identify the species.

FLN Agar provides a means to test for fluorescence, lactose oxidation and denitrification. Further differentiation can be obtained by testing for oxidase, lysine decarboxylation and fructose oxidation.[1]

Principles of the Procedure

To demonstrate the ability of isolates to produce fluorescin pigment, to reduce nitrate to nitrite to nitrogen gas and to oxidize lactose, medium B of King (Pseudomonas Agar P/Tech Agar)[2] was modified.[1] The ability of isolates to produce fluorescin and to reduce nitrate or nitrite to nitrogen gas are two important characteristics in differentiating certain *Pseudomonas* spp. (principally *P. aeruginosa*) from other nonfermentative bacilli. The inclusion of lactose and phenol red indicator in the medium permits the detection of acid formed from the oxidation of lactose and aids in identifying the strongly lactose-positive group of nonfermenters.[3]

Procedure

Specimens must first be isolated in pure culture on an appropriate medium. The isolate should be Gram-stained and examined to confirm that morphology is appropriate for the gram-negative bacilli.

Using a sterile inoculating needle, streak the slant surface and stab the butt with several colonies from the subculture medium. Incubate the tubes, with caps loosened, at 35°C for 18-24 hours.

If the isolate fails to grow, reincubate at 25-30°C for up to 1 week; examine daily for growth and pigment production. If pigmentation fails to develop after the initial 24 hours of incubation, reincubate the cultures at 22°C for 1 or more days.

Expected Results

Examine FLN Agar under UV light for fluorescin, a greenish-yellow fluorescent pigment in the colonies and surrounding medium. Observe butt of slant for presence of gas bubbles as evidence of denitrification. Yellow color on slant indicates oxidation of lactose.

References

1. Pickett, and Pedersen. 1968. Appl. Microbiol. *16*:1631.
2. King, Ward, and Raney. 1954. J. Lab. Clin. Med. *44*:301.
3. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.

Availability

BBL™ FLN Agar

Cat. No. 296363 Prepared Slants – Pkg. of 10

FTA Hemagglutination Buffer

Intended Use
FTA Hemagglutination Buffer (Phosphate Buffered Saline, pH 7.2) is used in the FTA-ABS test and other serological procedures as a diluent and for washing slide preparations.

Summary and Explanation
This phosphate buffered saline product is widely used in immunology as a diluent in serological procedures. It is referred to as FTA Hemagglutination Buffer because of its use in the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) test, which is a serological test for syphilis.

Principles of the Procedure
This phosphate buffered saline provides an isotonic environment for microorganisms during manipulation pursuant to the performance of serological test procedures.

User Quality Control

Identity Specifications
BBL™ FTA Hemagglutination Buffer

Dehydrated Appearance:	White, fine, homogeneous, free of extraneous material.
Solution:	0.923% solution, soluble in purified water. Solution is colorless, clear.
Prepared Appearance:	Colorless, clear.
Reaction of 0.923% Solution at 25°C:	pH 7.2 ± 0.1

Formula
BBL™ FTA Hemagglutination Buffer

Approximate Formula* Per Liter

Sodium Chloride	7.65 g
Disodium Phosphate	1,268.8 mg
Monosodium Phosphate	0.1 g
Monopotassium Phosphate	211.3 mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Dissolve 9.23 g of the powder in 1 L of purified water. DO NOT AUTOCLAVE.
2. Test samples of the finished product for performance.

Procedure
Refer to specific serological procedures for the use of this buffer.[1]

Reference
1. Larsen, Pope, Johnson and Kennedy (ed.). 1998. A manual of tests for syphilis, 9th ed. American Public Health Association, Washington, D.C.

Availability
BBL™ FTA Hemagglutination Buffer

Cat. No. 211248 Dehydrated – 500 g

Fildes Enrichment

Intended Use
Fildes Enrichment may be used to enrich a variety of media for the cultivation of various microorganisms.

User Quality Control

Identity Specifications
BBL™ Fildes Enrichment

Appearance:	Dark brown and hazy with fine dark brown sediment.

Cultural Response
BBL™ Fildes Enrichment

Prepare medium with added Fildes Enrichment. Inoculate with fresh cultures and incubate at 35 ± 2°C for 2 days with CO_2.

ORGANISM	ATCC™	RECOVERY
Haemophilus influenzae	10211	Good
Neisseria meningitidis	700344	Good

Summary and Explanation
Fildes Enrichment is a peptic digest of sheep blood used to enhance the growth of fastidious organisms. It may be added to Tryptic/**Trypticase**™ Soy Broth or Agar in a final concentration of 5% for cultivation of *Haemophilus influenzae*.[1,2]

Stokes and Willis both recommended that 5% Fildes Enrichment and 20% human serum be added to Nutrient Agar for Nagler plates for *C. perfringens* and *C. bifermentans*.[3,4]

Principles of the Procedure
Fildes Enrichment is a rich source of growth factors stimulatory to various microorganisms, including the X (hemin) and V (nicotinamide adenine dinucleotide, NAD) factors necessary for the growth of *H. influenzae*.

Formula
Fildes Enrichment is prepared by the action of the enzyme pepsin on defibrinated sheep blood.

Procedure

Fildes Enrichment is a ready-to-use solution. Many factors in Fildes Enrichment are heat labile. This enrichment cannot be heated and must be added aseptically in the proper amounts to media that have been autoclaved and cooled to 50-55°C.

Fildes Enrichment is usually employed in prepared media at a final concentration of 5% for optimal results. Some formulas may require higher or lower concentrations. Add Fildes Enrichment as required.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Fildes. 1920. Br. J. Exp. Pathol. *1*:129.
2. Parker and Hoeprich. 1962. Am. J. Clin. Pathol. *37*:319.
3. Stokes. 1960. Clinical bacteriology, 2nd ed. Edward Arnold, Ltd., London.
4. Willis. 1960. Anaerobic bacteriology in clinical medicine. Butterworth & Co., Ltd., London.

Availability

BBL™ Fildes Enrichment

Cat. No. 211866 Prepared Tubes (K Tubes), 5 mL – Pkg. of 10*

**Store at 2-8°C.*

Fletcher's Media
Fletcher Medium Base • Fletcher's Medium
Fletcher's Medium with 5-FU

Intended Use

Fletcher's Medium is an enriched, semisolid medium used for the cultivation of *Leptospira*.

Fletcher's Medium with 5-FU contains 5-fluorouracil for selective recovery and cultivation of *Leptospira* from clinical specimens.

Summary and Explanation

Leptospirosis is an acute, febrile disease caused by members of the genus *Leptospira*.[1,2] Direct culture of blood is the most reliable way to detect *Leptospira* during the first week of illness. After the first week of illness and for several months thereafter, leptospires may be isolated by direct culture of undiluted urine specimens. At autopsy, leptospires may be isolated from kidney and liver tissues as well as from blood and urine.

Fletcher developed an enriched medium for the cultivation of *Leptospira* from clinical specimens (urine, blood, kidney and liver tissues).[3] Peptone and a rabbit serum enrichment support the growth of leptospires.

When supplemented with 5-fluorouracil, the medium is recommended for urine and other specimens containing mixed microbial flora to provide selective inhibition of bacterial contaminants without inhibiting the growth of leptospires.[4]

Principles of the Procedure

Peptone and beef extract provide amino acids and other nitrogenous substances to support bacterial growth. Sodium chloride provides essential ions. A small amount of agar provides a semi-solid consistency, which helps in the detection of motile organisms.

The 5-fluorouracil is a fluorinated pyrimidine analog that inhibits bacterial contaminants without affecting the growth of *Leptospira*.

Formula

Difco™ Fletcher Medium Base

Approximate Formula* Per 920 mL		
Peptone	0.3	g
Beef Extract	0.2	g
Sodium Chloride	0.5	g
Agar	1.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 2.5 g of the powder in 920 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add 80 mL sterile normal rabbit serum at 56°C. Mix well.
5. Determine pH; if necessary, aseptically adjust to pH 7.9 ± 0.1 with 1N HCl or 1N NaOH.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Prepare the medium from Fletcher Medium Base per label directions and aseptically dispense into sterile screw-cap tubes in 5-7 mL amounts. Store at room temperature overnight. Inactivate the whole medium the day following its preparation by placing the tubes in a water bath at 56°C for 1 hour. Allow the medium to cool before inoculation.

Inoculate the medium with one or two drops of blood or urine per tube and distribute throughout the medium. Leptospires are most likely to be isolated from blood during the first week of illness. Thereafter, they are more likely to be isolated from urine. Both undiluted and 10-fold diluted urine specimens should be cultured because the undiluted urine may contain growth-inhibiting substances. Repeat the inoculation procedures to obtain optimal recovery of *Leptospira*, since they may be shed sporadically.

User Quality Control

Identity Specifications

Difco™ Fletcher Medium Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	0.25 g/92 mL solution, soluble in purified water upon boiling. Solution is very light amber, clear to very slightly opalescent.
Prepared Appearance:	Very light amber, very slightly opalescent.
Reaction of 0.25 g/92 mL Solution at 25°C:	pH 7.9 ± 0.1

Cultural Response

Difco™ Fletcher Medium Base

Prepare the medium per label directions. Inoculate with undiluted cultures and incubate at 30 ± 2°C for up to 5 days.

ORGANISM	ATCC™	INOCULUM	RECOVERY
Leptospira interrogans serotype australis	23605	0.1 mL	Good
Leptospira interrogans serotype canicola	23606	0.1 mL	Good
Leptospira kirschneri	23604	0.1 mL	Good

Leptospira may also be cultured from liver and kidney tissues. Aseptically macerate tissue specimens and inoculate using 1:1, 1:10 and 1:100 dilutions. Consult appropriate texts for detailed information about the processing and inoculation of tissues and other specimens.[1,2]

Incubate tubes in the dark at 25-30°C for up to 6 weeks.

Expected Results

Examine tubes for growth every 5-7 days. Growth occurs as a ringed-area (disk) 1-3 cm below the surface of the medium. The absence of a ringed area of growth does not necessarily mean leptospires are not present. Remove a small amount of growth from the disk area and examine microscopically (the Gram stain is not satisfactory). Microcolonies can be fixed with methanol and stained with Giemsa stain to show rod forms.[5]

Cultures should be held for up to 6 weeks before discarding as negative.

References

1. Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
2. Weyant, Bragg and Kaufmann. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Fletcher. 1927-28. Trans. Roy. Soc. Trop. Med. & Hyg. *21*:265.
4. Johnson and Rogers. 1964. J. Bacteriol. *87*:422.
5. Weinman. 1981. *In* Balows and Hausler (ed.), Diagnostic procedures for bacterial, mycotic and parasitic infections, 6th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Fletcher Medium Base

Cat. No. 298710 Dehydrated – 500 g

BBL™ Fletcher's Medium

Cat. No. 297242 Prepared Tubes (K Tubes), 5 mL – Pkg. of 10*

BBL™ Fletcher's Medium with 5-FU

SMWW

Cat. No. 297243 Prepared Tubes (K Tubes), 5 mL – Pkg. of 10*

**Store at 2-8°C.*

Flo Agar

(See Pseudomonas Agars)

Fluid A • Fluid D

Intended Use

Fluid A (peptone water) is used for diluting or rinsing when performing sterility testing. Fluid D (peptone water with polysorbate 80) is used for diluting or rinsing samples containing lecithin or oil when performing sterility testing.

Meets *United States Pharmacopeia* (*USP*) performance specifications.

Summary and Explanation

Pharmaceuticals, biologicals, medical devices or any material claiming to be sterile must be tested for sterility according to the procedures described in the compendia.[1,2] Sterility testing is performed using the membrane filtration or direct testing methods, depending upon sample type and size.

Fluid A and Fluid D are used for diluting or rinsing when performing sterility testing. These fluids aid in the complete rinsing of the membrane filter apparatus and are not toxic to microorganisms.

Fluid D contains polysorbate 80, which acts as a surfactant to break down the lecithin or oils present.

Principles of the Procedure

Fluid A and Fluid D contain peptic digest of animal tissue, which provides a source of nitrogen for bacteria. Polysorbate 80 in Fluid D is a surfactant.

Formulae

Difco™ Fluid A

Approximate Formula* Per Liter		
Peptic Digest of Animal Tissue	1.0	g

Difco™ Fluid D

Approximate Formula* Per Liter		
Peptic Digest of Animal Tissue	1.0	g
Polysorbate 80	1.0	mL

**Adjusted and/or supplemented as required to meet performance criteria.*

Procedure

Fluid A and Fluid D are provided as prepared, ready-to-use diluents in a variety of bottle sizes and closures. Consult appropriate references for detailed information and recommended procedures.[1,2]

Expected Results

Consult appropriate references for further information.[1,2]

User Quality Control

Identity Specifications

Difco™ Fluid A

Appearance:	Nearly colorless, clear solution.
Reaction of Solution at 25°C:	pH 7.1 ± 0.2

Difco™ Fluid D

Appearance:	Nearly colorless, clear solution.
Reaction of Solution at 25°C:	pH 7.1 ± 0.2

Toxicity Test

Difco™ Fluid A or Fluid D

Perform a toxicity test by inoculating duplicate tubes of Tryptic Soy Broth with the test organisms and adding 1 mL of Fluid A or Fluid D to one tube of each set. Incubate tubes at 20-25°C for up to 5 days. Good recovery in the tubes containing Fluid A or Fluid D indicates that the solution does not have antibacterial or antifungal properties and is suitable for use in appropriate procedures.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10-10²	Good
Candida albicans	10231	10-10²	Good
Kocuria rhizophila	9341	10-10²	Good

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Council of Europe. 2008. European pharmacopeia, 6th ed. Council of Europe, Strasbourg, France.

Availability

Difco™ Fluid A

EP USP

Cat. No.	292391	Prepared Bottles, 100 mL (combo cap) – Pkg. of 10
	290821	Prepared Bottles, 100 mL (serum) – Pkg. of 10
	290652	Prepared Bottles, 300 mL (septum screw cap) – Pkg. of 10
	299111	Prepared Bottles, 500 mL – Pkg. of 10
	292478	Prepared Bottles, 600 mL (septum screw cap) – Pkg. of 10

Europe

Cat. No.	254979	Prepared Bottles, 300 mL – Pkg. of 10
	257096	Prepared Bottles, 650 mL – Pkg. of 4
	257332	Prepared Bottles, 100 mL (infusion) – Pkg. of 25
	257324	Prepared Bottles, 400 mL – Pkg. of 10
	257330	Prepared Bottles, 700 mL – Pkg. of 4
	257263	Prepared Bottles, 300 mL (double bagged) – Pkg. of 10
	257262	Prepared Bottles, 300 mL (double bagged) – Pkg. of 4

Difco™ Fluid D

EP USP

Cat. No.	299447	Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10
	290831	Prepared Bottles, 100 mL (serum) – Pkg. of 10
	290662	Prepared Bottles, 300 mL (septum screw cap) – Pkg. of 10
	299110	Prepared Bottles, 500 mL – Pkg. of 10
	210049	Prepared Bottles, 600 mL (septum screw cap) – Pkg. of 10

Europe

Cat. No.	257299	Prepared Bottles, 500 mL – Pkg. of 10
	254978	Prepared Bottles, 300 mL (double bagged) – Pkg. of 10

Fluid Sabouraud Medium

(See Sabouraud Media)

Fluid Thioglycollate Media

(See Thioglycollate Media)

Folic AOAC Medium

Intended Use

Folic AOAC Medium is used for determining folic acid concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Folic AOAC Medium is prepared for use in the microbiological assay of folic acid according to the procedures of the Folic Acid Assay in the *Official Methods of Analysis of AOAC International*.[1] *Enterococcus hirae* ATCC™ 8043 is the test organism in this assay.

Principles of the Procedure

Folic Acid AOAC Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *E. hirae* ATCC 8043. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Folic AOAC Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	10.0	g
L-Asparagine	0.6	g
L-Tryptophan	0.2	g
L-Cysteine Hydrochloride	0.76	g
Dextrose	40.0	g
Adenine Sulfate	10.0	mg
Guanine Hydrochloride	10.0	mg
Uracil	10.0	mg
Xanthine	20.0	mg
p-Aminobenzoic Acid	1.0	mg
Pyridoxine Hydrochloride	4.0	mg
Thiamine Hydrochloride	400.0	µg
Calcium Pantothenate	800.0	µg
Nicotinic Acid	800.0	µg
Biotin	20.0	µg
Riboflavin	1.0	mg
Glutathione	5.2	mg
Polysorbate 80	0.1	g
Sodium Citrate	52.0	g
Dipotassium Phosphate	6.4	g
Magnesium Sulfate	0.4	g
Manganese Sulfate	20.0	mg
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilizing and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 11 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Distribute 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 5 minutes.

User Quality Control

Identity Specifications

Difco™ Folic AOAC Medium

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	5.5% (single strength) or 11.0% (double strength) solution, soluble in purified water upon boiling. Single strength solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear.
Reaction of 5.5% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Folic AOAC Medium

Prepare the medium per label directions. The medium supports the growth of *Enterococcus hirae* ATCC™ 8043 when prepared in single strength and supplemented with folic acid. The medium should produce a standard curve when tested using a folic acid reference standard at 0.0 to 10.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-18 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Procedure

Follow assay procedures as outlined in the reference.[1] It is essential that a standard curve be set up for each separate assay. Autoclaving and incubation conditions that can influence the standard curve readings cannot always be duplicated. The standard curve is obtained by using folic acid at levels of 0.0, 1, 2, 4, 6, 8 and 10 ng per assay tube (10 mL). Folic AOAC Medium may be used for both turbidimetric and titrimetric analysis. Turbidimetric readings should be taken after incubation at 35-37°C for 16-18 hours. Titrimetric determinations are best made following incubation at 35-37°C for 72 hours.

The folic acid required for the preparation of the standard curve may be prepared as follows:

A. Dissolve 50 mg dried folic acid in about 30 mL 0.01N NaOH and 300 mL purified water.
B. Adjust the pH reaction to 7.5 ± 0.5 with diluted HCl solution. Dilute to 500 mL with purified water.
C. Add 2 mL of the solution to 50 mL purified water. Adjust the pH reaction to 7.5 ± 0.5. Dilute to 100 mL with purified water. This yields a stock solution containing 2 µg folic acid per mL.
D. Prepare the stock solution fresh daily.

The standard solution for the assay is made by diluting 1 mL of this stock solution to 1 liter with purified water. This solution contains 2 ng folic acid per mL. Use 0.0, 0.5, 1, 2, 3, 4, and 5 mL per assay tube.

Some laboratories may wish to alter the concentration of folic acid recommended above for the standard curve. This is permissible if the concentration used is within the limits specified by AOAC.[1]

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

Reference

1. Horwitz (ed.). 2007. Official methods of analysis of AOAC international, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Folic AOAC Medium

AOAC

Cat. No. 212169 Dehydrated – 100 g*

**Store at 2-8°C.*

Folic Acid Assay Medium

Intended Use

Folic Acid Assay Medium is used for determining folic acid concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of medium are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Folic Acid Assay Medium is used in the microbiological assay of folic acid with *Enterococcus hirae* ATCC™ 8043 as the test organism. Folic Acid Assay Medium is prepared according to the formula described by Capps, Hobbs and Fox,[1] modified with sodium citrate instead of sodium acetate.

Principles of the Procedure

Folic Acid Assay Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *E. hirae* ATCC 8043. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Difco™ Folic Acid Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Citrate	20.0	g
L-Cystine	0.2	g
DL-Tryptophan	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Thiamine Hydrochloride	2.0	mg
Pyridoxine Hydrochloride	4.0	mg
Riboflavin	2.0	mg
Niacin	2.0	mg
Calcium Pantothenate	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Biotin	0.8	µg
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

User Quality Control

Identity Specifications

Difco™ Folic Acid Assay Medium

Dehydrated Appearance:	Off-white to very light beige, free-flowing, homogeneous.
Solution:	3.75% (single strength) or 7.5% (double strength) solution, soluble in purified water upon boiling for 2-3 minutes. Single strength solution is light amber, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear, may have a very slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Folic Acid Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Enterococcus hirae* ATCC™ 8043 when prepared in single strength and supplemented with folic acid. The medium should produce a standard curve when tested using a folic acid reference standard at 0.0 to 10.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Directions for Preparation from Dehydrated Product

1. Suspend 7.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Dispense in 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

Procedure

Prepare stock cultures of *E. hirae* ATCC 8043 by stab inoculation of Lactobacilli Agar AOAC. Incubate at 35-37°C for 24-48 hours. Store tubes in the refrigerator. Make transfers at monthly intervals. Prepare the inoculum for assay by subculturing a stock culture of *E. hirae* ATCC 8043 into a tube containing 10 mL of Lactobacilli Broth AOAC. After incubation at 35-37°C for 18-24 hours, centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 mL of sterile 0.85% saline. After the third wash, dilute the cell suspension 1:100 with sterile 0.85% saline. Use one drop of this latter suspension to inoculate each of the assay tubes.

It is essential that a standard curve be set up for each separate assay. Autoclaving and incubation conditions that influence the standard curve readings cannot always be duplicated. The standard curve is obtained by using folic acid at levels of 0.0, 2, 4, 6, 8 and 10 ng per 10 mL assay tube. Turbidimetric readings should be made after incubation at 35-37°C for 18-24 hours. Refrigerate tubes for 15-30 minutes to stop growth before reading.

Prepare the folic acid stock solution required for the standard curve as follows:

1. Dissolve 50 mg dried Folic Acid USP Reference Standard or equivalent in about 30 mL of 0.01N NaOH and 300 mL purified water.
2. Adjust to pH 7.5 ± 0.5 with diluted HCl solution. Add purified water to give a volume of 500 mL.
3. Add 2 mL of the solution from step 2 to 50 mL purified water. Adjust the pH to 7.5 ± 0.5 with HCl solution. Dilute to 100 mL with purified water to give a stock solution containing 2 µg folic acid per mL. Prepare the stock solution fresh daily.

Prepare the standard solution for the assay by diluting 1 mL of this stock solution in 1 liter with purified water. This solution contains 2 ng folic acid per mL. Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL per assay tube.

Following incubation, place the tubes in the refrigerator for 15-30 minutes to stop growth. The growth can be measured by a turbidimetric method and the curve constructed from the values obtained. The most effective assay range is between the levels of 2 and 10 ng folic acid per 10 mL tube.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

Reference

1. Capps, Hobbs and Fox. 1948. J. Bacteriol. *55*:869.

Availability

Difco™ Folic Acid Assay Medium

Cat. No. 231810 Dehydrated –100 g*

**Store at 2-8°C.*

Folic Acid Casei Medium

Intended Use

Folic Acid Casei Medium is used for determining folic acid concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Folic Acid Casei Medium is prepared for the microbiological assay of folic acid, particularly folic acid in serum. *Lactobacillus rhamnosus* ATCC™ 7469 is used as the test organism in this assay. Folic Acid Casei Medium is prepared according to the formulation described by Flynn et al.[1] and modified by Baker et al.[2] and Waters and Mollin.[3]

Total serum folic acid activity can vary depending on the disease state. It has been reported that normal subjects have a mean serum folic acid level of 9.9 ng per mL. Patients with uncomplicated pernicious anemia have a mean serum folic acid level of 16.6 ng per mL, while patients with megaloblastic anemia have levels less than 4.0 ng per mL.

User Quality Control

Identity Specifications

Difco™ Folic Acid Casei Medium

Dehydrated Appearance:	Off-white, homogeneous, with soft clumps.
Solution:	4.7% (single strength) soluble in purified water upon boiling 1-2 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Single-strength solution is very light amber, clear, may have a very slight precipitate.
Reaction of 4.7% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Folic Acid Casei Medium

Prepare the medium per label directions with the addition of 0.05% ascorbic acid. Dilute folic acid to produce a standard solution in folic acid buffer solution (consisting of per liter: monopotassium phosphate 10.65 g, dipotassium phosphate 3.744 g and ascorbic acid 1.0 g and having a final pH at 25°C of 6.1 ± 0.05). The medium supports the growth of *Lactobacillus rhamnosus* ATCC™ 7469 when prepared in single strength and supplemented with folic acid. The medium should produce a standard curve when tested using a folic acid reference standard at 0.0 to 1.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Principles of the Procedure

Folic Acid Casei Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. rhamnosus* ATCC 7469. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Difco™ Folic Acid Casei Medium

Approximate Formula* Per Liter		
Charcoal Treated Pancreatic Digest of Casein	10.0	g
Dextrose	40.0	g
Sodium Acetate	40.0	g
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
DL-Tryptophan	0.2	g
L-Asparagine	0.6	g
L-Cysteine Hydrochloride	0.5	g
Adenine Sulfate	10.0	mg
Guanine Hydrochloride	10.0	mg
Uracil	10.0	mg
Xanthine	20.0	mg
Polysorbate 80	0.1	g
Glutathione (reduced)	5.0	mg
Magnesium Sulfate (anhydrous)	0.2	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	15.0	mg
Riboflavin	1.0	mg
p-Aminobenzoic Acid	2.0	mg
Pyridoxine Hydrochloride	4.0	mg
Thiamine Hydrochloride	400.0	µg
Calcium Pantothenate	800.0	µg
Nicotinic Acid	800.0	µg
Biotin	20.0	µg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 9.4 g of the powder in 100 mL of purified water.
2. Add 50 mg ascorbic acid. Mix thoroughly.
3. Heat with frequent agitation and boil for 1-2 minutes to completely dissolve the powder.
4. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
5. Add standard or test samples.
6. Adjust tube volume to 10 mL with purified water.
7. Autoclave at 121°C for 5 minutes.

Procedure

Preparation of Stock Cultures and Inoculum

Prepare stock cultures of the test organism, *L. rhamnosus* ATCC 7469, by stab inoculation into prepared tubes of Lactobacilli Agar AOAC. Incubate the cultures at 35-37°C for 18-24 hours. Store cultures in the refrigerator at 2-8°C. Stock transfers are made at monthly intervals.

Prepare the inoculum for assay by subculturing from a stock culture of *L. rhamnosus* into a tube containing 10 mL prepared Micro Inoculum Broth. Incubate at 35-37°C for 16-18 hours. Under aseptic conditions, centrifuge the tubes to sediment the cells and decant the supernatant. Wash the cells in 10 mL sterile single-strength Folic Acid Casei Medium. Resediment the cells by centrifuging aseptically and decant the supernatant. Repeat washing two more times. After the third washing, resuspend the cells in 10 mL sterile single-strength medium and dilute 1 mL with 99 mL of the same medium. One drop of this suspension is used to inoculate each of the assay tubes. Read the growth response of the assay tubes turbidimetrically after 18-24 hours incubation at 35-37°C. (Some laboratories use 0.85% saline instead of the single-strength basal medium to wash and dilute the inoculum.)

Preparation of the Standard

It is essential that a standard curve be constructed for each separate assay. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated. The standard curve may be obtained by using folic acid at levels of 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ng per assay tube (10 mL).

The folic acid required for preparation of the standard curve may be prepared as follows:

Dissolve 50 mg dried folic acid in about 30 mL 0.01N NaOH and 300 mL purified water. Adjust to pH 7-8 with 0.05N HCl and dilute to 500 mL with purified water. Dilute 10 mL of this solution with 500 mL purified water. Further dilute 1 mL in 1 L purified water to make a stock solution containing 2 ng per mL folic acid. Prepare the standard solution containing 0.2 ng per mL folic acid by diluting 10 mL of stock solution with 90 mL of folic buffer solution (consisting of per liter: monopotassium phosphate 10.656 g, dipotassium phosphate 3.744 g and ascorbic acid 1.0 g and having a final pH at 25°C of 6.1 ± 0.05). Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL per assay tube.

Prepare the stock solution fresh daily.

Preservation of Serum Specimens

1. Allow the blood specimen to clot and the serum to separate from the clot.
2. Aspirate the serum into a clean dry tube and centrifuge to remove any cells that may be present. Avoid hemolysis. Dispense 5 mL of each serum sample into clean dry test tubes and add 25 mg ascorbic acid to each tube.
3. If the test is not begun immediately, place tubes in a freezer and hold below -20°C.

Preparation of Serum Specimen

1. Thaw the serum containing ascorbic acid.
2. Add 5 mL of the uniform sample to 45 mL rehydrated folic buffer solution (see "Preparation of the Standard").
3. Incubate the serum-buffer solution at 37°C for 90 minutes. Autoclave the incubated mixture at 121°C for 2.5 minutes.
4. Remove the coagulated protein by centrifuging and transfer the clear supernatant to a clean dry tube. The clear solution is the sample to use in the folic acid assay.

Procedure for Total Folic Acid

1. Use 0.5, 1.0, 1.5 mL or other volumes of the prepared serum extracts as described above.
2. Fill each assay tube with 5 mL of rehydrated Folic Acid Casei Medium and sufficient purified water to give a total volume of 10 mL per tube.
3. Autoclave tubes at 121°C for 5 minutes.
4. Add 1 drop of inoculum described under "Preparation of Stock Cultures and Inoculum" to each assay.
5. Incubate at 35-37°C for 18-24 hours. Tubes are refrigerated for 15-30 minutes to stop growth before reading turbidimetrically.

Expected Results

The amount of folic acid in the test samples can be determined by interpolating the results with the values obtained on the standard curve, taking into consideration the dilutions of the samples.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. Flynn, Williams, O'Dell and Hogan. 1951. Anal. Chem. *23*:180.
2. Baker, Herbert, Frank, Pasher, Hunter, Wasserman and Sobotka. 1959. Clin. Chem. *5*:275.
3. Waters and Molin. 1961. J. Clin. Pathol. *14*:335.

Availability

Difco™ Folic Acid Casei Medium

Cat. No. 282210 Dehydrated – 100 g*

**Store at 2-8°C.*

Fraser Broth Base
Fraser Broth Supplement

Intended Use

Fraser Broth Base is used with Fraser Broth Supplement in selectively enriching and detecting *Listeria*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported foodborne outbreak of listeriosis was in 1985,[3] and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has indicated that the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, silage and river water.[6]

Fraser Broth Base and Fraser Broth Supplement are based on the formulation of Fraser and Sperber.[7] The medium is use in the rapid detection of *Listeria* from food[8] and environmental samples. Many common food contaminants such as streptococci, enterococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* interfere with the isolation of *Listeria monocytogenes*.[9]

Listeria species grow on laboratory media over a pH range of 4.4-9.6, and can survive in foods of similar acidity for days or weeks.[10] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Principles of the Procedure

Peptones, beef extract and yeast extract provide nitrogen, vitamins and minerals. Sodium phosphate and potassium phosphate are buffering agents. Differentiation is aided by including ferric ammonium citrate in the final medium. Since all *Listeria* species hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the medium by cultures containing esculin-hydrolyzing bacteria is the result of the formation of 6,7-dihydroxycoumarin that reacts with the ferric ions.[7]

Selectivity is provided by the presence of lithium chloride, nalidixic acid and acriflavine in the formula. The high salt tolerance of *Listeria* is used as a means to inhibit growth of enterococci.

Formulae

Difco™ Fraser Broth Base

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	5.0	g
Beef Extract	5.0	g
Yeast Extract	5.0	g
Sodium Chloride	20.0	g
Disodium Phosphate	9.6	g
Monopotassium Phosphate	1.35	g
Esculin	1.0	g
Nalidixic Acid	0.02	g
Acriflavine HCl	24.0	mg
Lithium Chloride	3.0	g

Difco™ Fraser Broth Supplement

Per 10 mL Vial		
Ferric Ammonium Citrate	0.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 55 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Aseptically add 10 mL Fraser Broth Supplement. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To isolate *Listeria monocytogenes* from processed meats and poultry, the following procedure is recommended by the U.S. Department of Agriculture.[8]

1. Add 25 g of test material to 225 mL of UVM Modified Listeria Enrichment Broth and mix or blend thoroughly.
2. Incubate for 20-24 hours at 30°C.
3. Transfer 0.1 mL of the incubated broth to Fraser Broth. Incubate at 35°C for 26 ± 2 hours.
4. At 24 and 48 hours, streak the Fraser Broth culture to Modified Oxford Agar.
5. Incubate the Modified Oxford plates at 35°C for 24-48 hours.

Expected Results

1. Examine agar plates for suspect colonies. For further identification and confirmation of *Listeria* spp., consult appropriate references.[8,10,11]
2. Rapid slide and macroscopic tube tests can be used for definitive serological identification.

User Quality Control

Identity Specifications

Difco™ Fraser Broth Base

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution:	5.5% solution, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent with a fine precipitate.
Prepared Appearance:	Medium amber, clear to slightly opalescent with a fine precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Fraser Broth Supplement

Solution Appearance: Dark brown solution.

Cultural Response

Difco™ Fraser Broth Base and Fraser Broth Supplement

Prepare the medium per label directions. Add Fraser Broth Supplement. Inoculate and incubate at 35 ± 2°C and read for growth and blackening at 18-24 and 42-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ESCULIN REACTION
Enterococcus faecalis	29212	10^3-2×10^3	Marked to complete inhibition	–
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition	–
Listeria monocytogenes	19114	10^2-10^3	Good	+
Listeria monocytogenes	19115	10^2-10^3	Good	+
Staphylococcus aureus	25923	10^3-2×10^3	Marked to complete inhibition	–

Uninoculated Tube

Listeria monocytogenes ATCC™ 19114

Limitations of the Procedure

1. Since *Listeria* species other than *L. monocytogenes* can grow on these media, an identification of *Listeria monocytogenes* must be confirmed by biochemical and serological testing.[11]
2. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

References

1. Murray, Webb and Swann. 1926. J. Pathol. Bacteriol. *29*:407.
2. Monk, Clavero, Beuchat, Doyle and Brackett. 1994. J. Food Prot. *57*:969.
3. Wehr. 1987. J. Assoc. Off. Anal. Chem. *70*:769.
4. Bremer and Osborne. 1995. J. Food Prot. *58*:604.
5. Grau and Vanderlinde. 1992. J. Food Prot. *55*:4.
6. Patel, Hwang, Beuchat, Doyle and Brackett. 1995. J. Food Prot. *58*:244.
7. Fraser and Sperber. 1988. J. Food Prot. *51*:762.
8. Lee and McClain. 1994. Laboratory Communication No. 57 (revised February 8, 1994). Food Safety and Inspection Service, Microbiology Division, USDA, Bethesda, Md.
9. Kramer and Jones. 1969. J. Appl. Bacteriol. *32*:381.
10. Ryser and Donnelly. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
11. Bille, Rocourt and Swaminathan. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Fraser Broth Base

CCAM COMPF ISO USDA

Cat. No. 211767 Dehydrated – 500 g
211766 Dehydrated – 2 kg

Difco™ Fraser Broth Supplement

CCAM COMPF ISO USDA

Cat. No. 211742 Vial – 6 × 10 mL*

**Store at 2-8°C.*

GC Medium Base • Supplement B • Supplement VX IsoVitaleX™ Enrichment • Hemoglobin • VCN Inhibitor VCNT Inhibitor • VCA Inhibitor • VCAT Inhibitor

Intended Use

Difco™ GC Medium Base is used with various additives in isolating and cultivating *Neisseria gonorrhoeae* and other fastidious microorganisms.

Difco Supplement B with Reconstituting Fluid B, **Difco** Supplement VX with Reconstituting Fluid VX and **BBL**™ **IsoVitaleX**™ Enrichment with Rehydrating Fluid are used for supplementing media to culture fastidious microorganisms, particularly *Neisseria gonorrhoeae* and *Haemophilus influenzae*.

BBL Hemoglobin products are used in preparing microbiological culture media.

BBL VCN Inhibitor, VCNT Inhibitor, VCA Inhibitor and VCAT Inhibitor are lyophilized preparations containing inhibitory agents to be used in selective media for culturing *N. gonorrhoeae* and *N. meningitidis*.

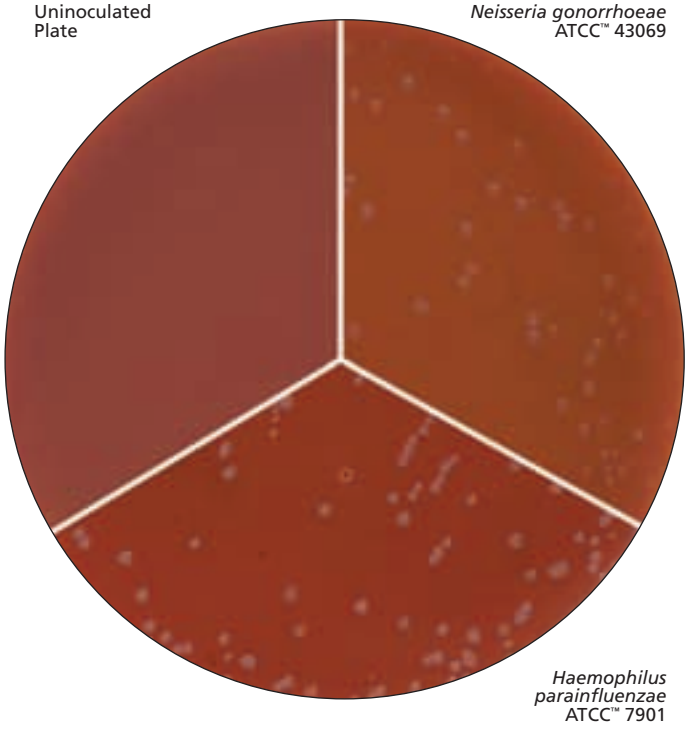
Summary and Explanation

In 1945, Johnston[1] described a medium that could successfully produce colonies of *N. gonorrhoeae* in 24 rather than 48 hours. The accelerated growth rates were primarily due to the decreased agar content (solidity) of the medium. GC Medium Base was introduced in 1947 with reduced agar content. While investigating the growth rate of some gonococcal strains, a medium containing the growth factors glutamine and cocarboxylase was found to improve recovery.[2,3] From this discovery, Supplement B, a yeast concentrate, was developed. In a comparative study[4] of 12 different media, an enriched chocolate agar prepared with GC Medium Base, Hemoglobin and Supplement B proved superior for isolating *N. gonorrhoeae*. **Difco** VX Supplement and **BBL™ IsoVitaleX™** Enrichment are chemically defined supplements developed to replace the yeast concentrate additive.

Difco Supplement B is a yeast concentrate for use in supplementing media for growth of microorganisms with exacting nutritional requirements. It is recommended for use in the preparation of chocolate agar described by Christensen and Schoenlein.[5]

Difco Supplement VX and **BBL™ IsoVitaleX™** Enrichment are lyophilized concentrates. These supplements are recommended for enriching GC Agar media, Proteose No. 3 Agar and the selective agars for the isolation of pathogenic *Neisseria*.

Hemoglobin, an autoclavable preparation of beef blood, provides hemin, which is required by *Haemophilus* species and enhances growth of *Neisseria* species.



In 1964, Thayer and Martin[6] formulated a selective medium incorporating the antibiotics polymyxin B and ristocetin into GC Agar with added hemoglobin and Supplement B. Thayer and Martin[7] improved their medium by replacing the two original antibiotics with a new antimicrobial solution of colistin, vancomycin and nystatin (VCN). In 1971, Martin and Lester[8] improved the new Thayer-Martin medium by incorporating an additional antibiotic, trimethoprim lactate (T), into the formulation (VCNT). This improved medium is called Modified Thayer-Martin (MTM) Medium.[9] VCN and VCNT are used in the preparation of Thayer-Martin and Modified Thayer-Martin agars, respectively.

Martin and Lewis[10] further improved the selectivity of MTM by increasing the concentration of vancomycin from 3.0 µg/mL to 4.0 µg/mL for greater inhibition of gram-positive bacteria and replacing nystatin with anisomycin (VCA/VCAT) for greater inhibition of yeasts; this is known as Martin-Lewis (ML) Agar Medium. Transgrow Medium bottles and Gono-Pak and JEMBEC™* plates are chocolate agar-based transport medium systems that can incorporate these formulations.[11]

**JEMBEC is a trademark of Miles Scientific.*

Principles of the Procedure

Peptones provide nitrogen, vitamins and amino acids. Corn starch absorbs any toxic metabolites that are produced; dibasic and monobasic potassium phosphates buffer the medium. Sodium chloride maintains osmotic balance. Agar is the solidifying agent.

Chocolate Agar is prepared from GC agar medium base with the addition of 2% Hemoglobin. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* and enhanced growth of *Neisseria*.

The growth rate of *Neisseria* and *Haemophilus* is improved with the addition of 1% nutritive enrichment, providing the growth factors glutamine and cocarboxylase. Supplement B contains yeast concentrate, glutamine, coenzyme (V factor), cocarboxylase, hematin (X factor) and growth factors. Supplement VX is a defined lyophilized concentrate of essential growth factors; i.e., vitamins, amino acids, coenzymes, dextrose and other factors to improve the growth of *Haemophilus* and *Neisseria* species. **IsoVitaleX** Enrichment provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

Antimicrobial agents are used as inhibitors in the selective media, Thayer-Martin, Modified Thayer-Martin and Martin Lewis agars and Transgrow Medium. Colistin inhibits gram-negative bacteria, vancomycin inhibits gram-positive contaminants, nystatin suppresses the growth of yeasts, anisomycin provides improved inhibition of *Candida albicans*, an organism that has been shown to inhibit *N. gonorrhoeae*,[12,13] and trimethoprim lactate suppresses the swarming of *Proteus* species.

Formulae

Difco™ GC Medium Base

Approximate Formula* Per Liter

Proteose Peptone No. 3	15.0	g
Corn Starch	1.0	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.0	g
Sodium Chloride	5.0	g
Agar	10.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

BBL™ Hemoglobin

An autoclavable preparation of beef blood. The 2% solution is ready for use in the preparation of media for the cultivation of fastidious organisms.

Difco™ Supplement B

Processed to preserve both the thermolabile and thermostable growth accessory factors of fresh yeast, it contains glutamine, coenzyme (V factor), cocarboxylase and other growth factors, as well as hematin (X factor).

Difco™ Supplement VX

Approximate Formula* Per 10 mL Vial

Adenine Sulfate	10.0	mg
p-Aminobenzoic Acid	0.25	mg
Cocarboxylase	2.0	mg
L-Cysteine HCl	259.0	mg
L-Cystine	11.0	mg
Nicotinamide Adenine Dinucleotide	3.5	mg
Ferric Citrate	0.3	mg
L-Glutamine	200.0	mg
Guanine HCl	0.3	mg
Thiamine HCl	0.06	mg
Vitamin B_{12} (Cyanocobalamin)	0.2	mg
Dextrose	1.0	g

BBL™ IsoVitaleX™ Enrichment

Approximate Formula* Per Liter

Adenine	1.0	g
p-Aminobenzoic Acid	13.0	mg
L-Cysteine Hydrochloride	25.9	g
L-Cystine	1.1	g
Nicotinamide Adenine Dinucleotide	0.25	g
Ferric Nitrate	0.02	g
L-Glutamine	10.0	g
Guanine Hydrochloride	0.03	g
Thiamine Hydrochloride	3.0	mg
Vitamin B_{12}	0.01	g
Dextrose	100.0	g
Thiamine Pyrophosphate	0.1	g

BBL™ VCN Inhibitor

Formula Per 1 mL Vial

Vancomycin	300	µg
Colistin	750	µg
Nystatin	1,250	units

BBL™ VCNT Inhibitor

Formula Per 1 mL Vial

Vancomycin	300	µg
Colistin	750	µg
Nystatin	1,250	units
Trimethoprim Lactate	500	µg

BBL™ VCA Inhibitor

Formula Per 1 mL Vial

Vancomycin	400	µg
Colistin	750	µg
Anisomycin	2.0	mg

User Quality Control

Identity Specifications

Difco™ GC Medium Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is light to medium amber, opalescent, may have a slight precipitate, "ground glass" appearance.
Prepared Appearance:	With Hemoglobin and Supplement, chocolate brown, opaque.
Reaction of 3.6% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Supplement B with Reconstituting Fluid

Lyophilized Appearance:	Tan to reddish brown, lyophilized powder or cake.
Rehydrated Appearance:	Medium to dark amber, very to slightly opalescent.
Reconstituting Fluid Appearance:	Colorless, clear liquid.

Difco™ Supplement VX with Reconstituting Fluid

Lyophilized Appearance:	Pink powder or cake.
Rehydrated Appearance:	Pink, clear.
Reconstituting Fluid Appearance:	Colorless, clear solution.

BBL™ Hemoglobin Solution 2%

Solution Appearance:	Dark chocolate brown with fine, very dark brown sediment, opaque.

BBL™ Hemoglobin, Bovine, Freeze-Dried

Dehydrated Appearance:	Coarse powder to crystals, free of extraneous material, medium to medium dark brown to red brown to black brown.
Solution:	2% solution, soluble in purified water.
Solution Appearance:	2% solution, medium to dark medium, brown to red brown to black brown, opaque.

BBL™ IsoVitaleX™ Enrichment with Diluent

Lyophilized Appearance:	Pink cake.
Rehydrated Appearance:	Light pink to pink tan, clear to trace hazy.
Diluent Appearance:	Colorless, clear solution.

BBL™ VCN Inhibitor

Lyophilized Appearance:	Pale yellow, dry cake.
Rehydrated Appearance:	Trace yellow-green and hazy containing a moderate amount of minute yellow particles.

BBL™ VCNT Inhibitor

Lyophilized Appearance:	Pale green, trace yellow.
Rehydrated Appearance:	Trace yellow, hazy, contains moderate amount of minute yellow particles.

BBL™ VCA Inhibitor

Lyophilized Appearance:	White to off-white.
Rehydrated Appearance:	Clear and colorless.

BBL™ VCAT Inhibitor

Lyophilized Appearance:	White to off-white, complete and dry, with scum-like disk evident.
Rehydrated Appearance:	Colorless, clear to moderately hazy.

Cultural Response

Difco™ GC Medium Base, Hemoglobin and Supplement B or Supplement VX or IsoVitaleX™ Enrichment

Prepare Chocolate Agar with GC Medium Base per label directions. Inoculate and incubate at 35 ± 2°C under 3-5% CO_2 for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Haemophilus influenzae	10211	30-300	Good
Neisseria gonorrhoeae	43069	30-300	Good

Difco™ GC Medium Base, Hemoglobin and Supplement B or Supplement VX or IsoVitaleX™ Enrichment and VCN Inhibitor or VCNT Inhibitor

Prepare Thayer-Martin Medium or Modified Thayer-Martin Medium with GC Medium Base per label directions, supplemented with VCN or VCNT Inhibitor, respectively. Inoculate and incubate at 35 ± 2°C under 3-5% CO_2 for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
*Candida albicans**	60193	30-300	Partial inhibition
Neisseria gonorrhoeae	43069	30-300	Good
*Neisseria meningitidis**	13090	30-300	Good
*Proteus mirabilis**	43071	30-300	Partial inhibition
Staphylococcus epidermidis	12228	30-300	Marked to complete inhibition

**Only used on VCNT-containing medium.*

Difco™ GC Medium Base, Hemoglobin and Supplement B or Supplement VX or IsoVitaleX™ Enrichment and VCA Inhibitor or VCAT Inhibitor

Prepare Martin-Lewis Agar with GC Medium Base according to label directions, supplemented with VCA Inhibitor or VCAT Inhibitor. Inoculate and incubate at 35 ± 2°C under 3-5% CO_2 for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Candida albicans	60193	30-300	Partial inhibition
Neisseria gonorrhoeae	43069	30-300	Good
*Neisseria meningitidis**	13090	30-300	Good
*Proteus mirabilis**	43071	30-300	Partial inhibition
Staphylococcus epidermidis	12228	30-300	Partial inhibition

**Only used on VCAT-containing medium.*

BBL™ VCAT Inhibitor

Formula Per 1 mL Vial

Vancomycin	400	µg
Colistin	750	µg
Anisomycin	2.0	mg
Trimethoprim Lactate	500	µg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Supplement B
Difco™ Supplement VX

1. Aseptically rehydrate Supplement B and Supplement VX with 10 mL or 100 mL of the corresponding Reconstituting Fluid, as appropriate.
2. Rotate the vial to dissolve completely.

BBL™ IsoVitaleX™ Enrichment

1. Reconstitute each lyophilized vial by aseptically transferring with a sterile syringe and needle the accompanying diluent.
2. Shake to assure complete solution.

BBL™ Hemoglobin

1. Dissolve 10 g of Hemoglobin powder for each L of medium desired in 1/2 volume of cold purified water.
2. Autoclave solution at 121°C for 15 minutes.
3. Cool to 45-50°C and combine with 1/2 volume of autoclaved agar base solution (such as GC agar medium base).
4. Mix thoroughly before dispensing into appropriate containers.

BBL™ Hemoglobin 2%

Shake the bottle to resuspend any sedimented hemoglobin before use.

BBL™ VCN Inhibitor, VCNT Inhibitor, VCA Inhibitor, VCAT Inhibitor

1. Restore each lyophilized vial by aseptically adding the appropriate amount of sterile purified water, as indicated on the label.
2. Rotate the vial to dissolve completely.

Chocolate Agar

1. Suspend 7.2 g of GC base medium in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 mL Hemoglobin Solution 2%.
5. Aseptically add 2 mL Supplement B or Supplement VX or **IsoVitaleX** Enrichment.
6. Dispense into sterile Petri dishes or tubes as desired.
7. Test samples of the finished product for performance using stable, typical control cultures.

Thayer-Martin Agar

1. Suspend 7.2 g of GC base medium in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 mL Hemoglobin Solution 2%.
5. Aseptically add 2 mL Supplement B or Supplement VX or **IsoVitaleX** Enrichment.
6. Aseptically add 2 mL rehydrated VCN Inhibitor to the medium.
7. Dispense into sterile Petri dishes.
8. Test samples of the finished product for performance using stable, typical control cultures.

Modified Thayer-Martin Medium

1. Suspend 7.2 g of GC base medium in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 mL Hemoglobin Solution 2% to the medium.
5. Aseptically add 2 mL **IsoVitaleX** Enrichment.
6. Aseptically add 2 mL rehydrated VCNT Inhibitor to the medium.
7. Dispense into sterile Petri dishes.
8. Test samples of the finished product for performance using stable, typical control cultures.

Martin-Lewis Agar

1. Suspend 7.2 g of GC base medium in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 mL Hemoglobin Solution 2%.
5. Aseptically add 2 mL **IsoVitaleX** Enrichment.
6. Aseptically add 2 mL rehydrated VCAT Inhibitor to the medium.
7. Dispense into sterile Petri dishes.
8. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the isolation and identification of *Neisseria* and *Haemophilus* species, consult the procedures outlined in the references.[14-16]

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. GC Medium Base is intended for use with supplementation. Although certain diagnostic tests may be performed directly on the medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
2. Improper specimen collection, environment, temperature, CO_2 level, moisture and pH can adversely affect the growth and viability of the organisms.
3. Inactivation or deterioration of antibiotics in selective media may allow growth of contaminants.
4. GC Medium Base has sufficient buffering capacity to offset the very low pH of the small amount of nutritive enrichments added. However, the pH of some media may have to be adjusted with 1% NaOH after the addition of these enrichments.

References

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2. Lankford, Scott, Cox and Cooke. 1943. J. Bacteriol. *45*:321.
3. Lankford and Snell. 1943. J. Bacteriol. *45*:421.
4. Carpenter, Bucca, Buck, Casman, Christensen, Crowe, Drew, Hill, Lankford, Morton, Peizer, Shaw and Thayer. 1949. Am. J. Syphil. Gonorrh. Vener. Dis. *33*:164.
5. Christensen and Schoenlein. 1947. Annu. Meet. Calif. Public Health Assoc.
6. Thayer and Martin. 1964. Public Health Rep. *79*:49.
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8. Martin and Lester. 1971. HSMHA Health Rep. *86*:30.
9. Martin, Armstrong and Smith. 1974. Appl. Microbiol. *27*:802.
10. Martin and Lewis. 1977. Public Health Rep. *35*:53.
11. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
12. Hipp, Lawton, Chen and Gaafar. 1974. Appl. Microbiol. *27*:192.
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15. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
16. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.

Availability

Difco™ GC Medium Base

Cat. No.	228950	Dehydrated – 500 g
	228920	Dehydrated – 2 kg
	228930	Dehydrated – 10 kg

BBL™ Hemoglobin Solution 2%

Cat. No.	211874	10 × 100 mL*

BBL™ Hemoglobin, Bovine, Freeze-Dried

Cat. No.	212392	Dehydrated – 500 g

Difco™ Supplement B

Cat. No.	227610	Lyophilized – 6 × 10 mL with Reconstituting Fluid*
	227620	Lyophilized – 100 mL with Reconstituting Fluid*

Difco™ Supplement VX

Cat. No.	233541	Lyophilized – 6 × 10 mL with Reconstituting Fluid*
	233542	Lyophilized – 100 mL with Reconstituting Fluid*

BBL™ IsoVitaleX™ Enrichment

Cat. No.	211875	Lyophilized – 5 × 2 mL with Diluent*
	211876	Lyophilized – 5 × 10 mL with Diluent*

BBL™ VCN Inhibitor

Cat. No.	212227	Lyophilized – 10 × 2 mL**
	212228	Lyophilized – 10 × 10 mL**

BBL™ VCNT Inhibitor

Cat. No.	212408	Lyophilized – 10 × 10 mL**

BBL™ VCA Inhibitor

Cat. No.	212269	Lyophilized – 10 × 10 mL**

BBL™ VCAT Inhibitor

Cat. No.	212404	Lyophilized – 10 × 10 mL**

**Store at 2-8°C.*
***Store at -20° to + 8°C.*

GC II Agar with IsoVitaleX™ Enrichment

Intended Use

GC II Agar with **IsoVitaleX**™ Enrichment is used for antimicrobial disc diffusion susceptibility testing of *Neisseria gonorrhoeae*.

Summary and Explanation

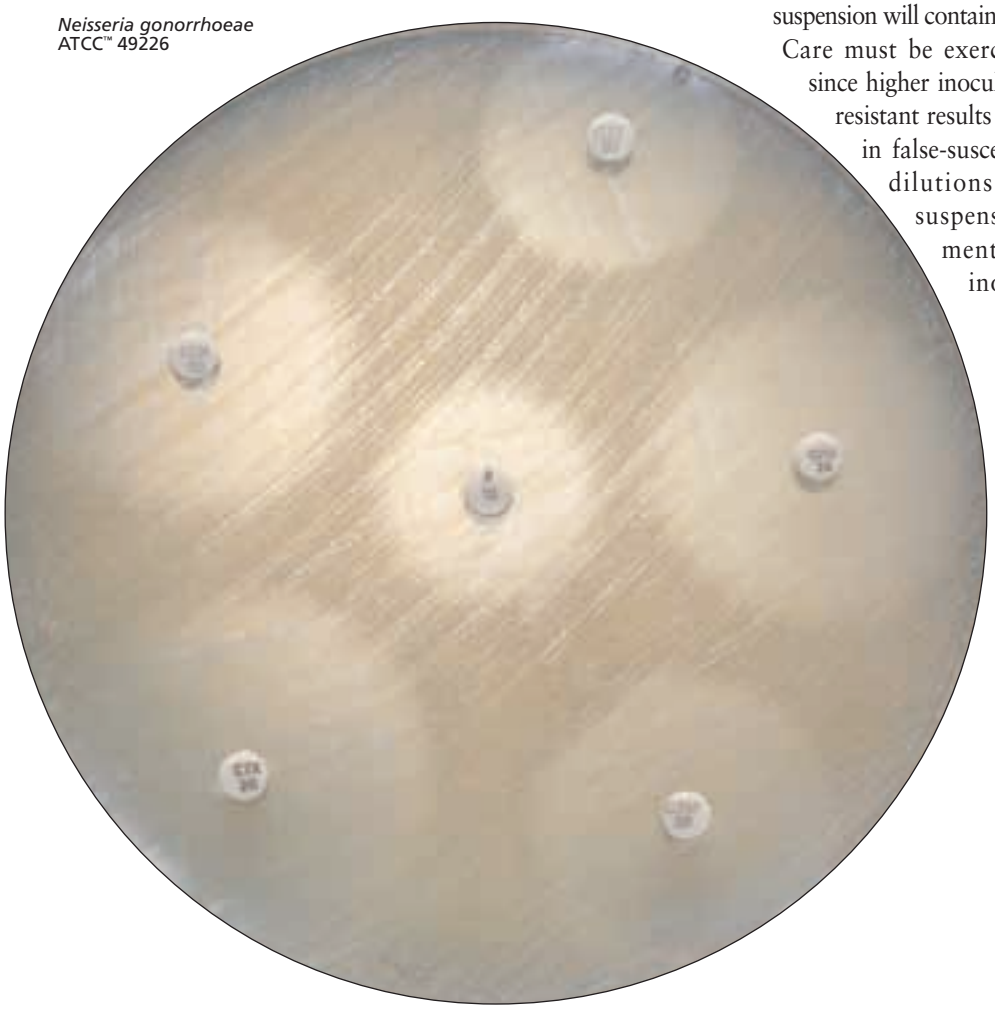
Because of the growing problem of resistance of gonococci to penicillin and other antimicrobial agents, the Centers for Disease Control and Prevention (CDC) issued, in 1987, policy guidelines for the detection, management and control of antibiotic-resistant *N. gonorrhoeae*.[1] The medium recommended for the disc diffusion method of antimicrobial susceptibility testing was GC agar base with a defined supplement, such as **BBL**™ **IsoVitaleX**™ Enrichment.

The Clinical and Laboratory Standards Institute (CLSI) also recommends the use of GC Agar with a 1% defined supplement (equivalent to **IsoVitaleX** Enrichment) for antimicrobial disc diffusion susceptibility testing of *N. gonorrhoeae*.[2,3]

Principles of the Procedure

GC II Agar Base contains casein and meat peptones as sources of nutrients, phosphate buffer to control pH and corn starch to neutralize toxic fatty acids that may be present in the agar. **IsoVitaleX** Enrichment is a defined supplement that provides V factor (nicotinamide adenine dinucleotide, NAD), vitamins, amino acids, coenzymes, dextrose, ferric ions and other factors that improve the growth of pathogenic *Neisseria*.

Neisseria gonorrhoeae
ATCC™ 49226



Procedure

The direct colony suspension procedure should be used when testing *N. gonorrhoeae*. Observe aseptic techniques. Agar surfaces should be smooth and moist but without excessive moisture.

1. Prepare a Gram stain and perform an oxidase test before starting susceptibility testing to confirm culture purity and to confirm tentative identification of *N. gonorrhoeae*.
2. Use several well-isolated colonies taken directly from an overnight chocolate agar plate as the source of the inoculum.
3. A rapid β-lactamase test (**Cefinase**™ disc) should be utilized to assess the clinical utility of penicillin. This rapid test provides the clinician with valuable antimicrobial susceptibility information sooner than the disc diffusion test.
4. Using colonies taken directly from an overnight chocolate agar plate, prepare a suspension of the test organism in Mueller Hinton Broth, Mueller Hinton II Broth or 0.9% saline and dilute with more broth or saline to a turbidity equivalent to that of the 0.5 McFarland standard. This suspension will contain approximately 1 to 2 × 10^6 CFU/mL. Care must be exercised in preparing this suspension since higher inoculum concentration may lead to false-resistant results and too light an inoculum can result in false-susceptible results. Periodically perform dilutions and plate counts of inoculum suspensions to confirm that the adjustment method employed produces an inoculum containing approximately 1 to 2 × 10^6 CFU/mL.
5. Within 15 minutes of adjusting the turbidity of the inoculum, dip a sterile swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
6. Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streakings to obtain even inoculation.
7. Replace the lid of the plate and hold the plate at room temperature for 3-5 minutes, but no longer than 15 minutes, to allow any surface moisture to be absorbed before applying the drug-impregnated discs.

8. Apply the discs by means of an antimicrobial disc dispenser, using aseptic precautions. Most antimicrobial agents produce larger zones of inhibition when tested against *N. gonorrhoeae* compared with other organisms; therefore, no more than 9 discs per 150 mm plate are recommended. After discs have been placed on the agar, tamp them with a sterile needle or forceps to make complete contact with the medium surface. This step is not necessary if the discs are deposited using the **Sensi-Disc**™ Self Tamping 12-Place Dispenser (tampers will not descend from holes lacking cartridges).
9. Within 15 minutes after the discs are applied, invert the plates and incubate for 20-25 hours at 35°C in an aerobic atmosphere enriched with 5-7% carbon dioxide.

Expected Results

1. Examine the plates after 20-24 hours of incubation. A confluent "lawn" of growth should be obtained. If only isolated colonies grow, the inoculum was too light and the test should be repeated.
2. Measure the diameter of the zone of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using calipers, a ruler, or a template prepared for this purpose. The measuring device is held on the back of the Petri plate, which is held over a black, non-reflecting background and illuminated from above. The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies that can be detected with difficulty near the edge of the obvious zone of inhibition.
3. Refer to the Zone Diameter Interpretive Standards in the CLSI publication for interpretation of results obtained with clinical isolates of *N. gonorrhoeae*.[3] Results may be reported as resistant, intermediate or susceptible depending on the zone diameters obtained. Organisms testing positive for β-lactamase production should be considered resistant to penicillin regardless of the zone diameters obtained.

Consult references for additional information.[4-6]

References

1. Centers for Disease Control. 1987. Antibiotic-resistant strains of *Neisseria gonorrhoeae*: policy guidelines for detection, management, and control. Morbid. Mortal. Weekly Rep. *36*(Suppl.):1S.
2. Clinical and Laboratory Standards Institute. 2006. Approved standard: M2–M9. Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.
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4. Neumann, Sahm, Thornsberry and McGowan. 1991. Cumitech 6A, New developments in antimicrobial agent susceptibility testing: a practical guide. Coordinating ed., McGowan. American Society for Microbiology, Washington D.C.
5. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington. D.C.

Availability

BBL™ GC II Agar with IsoVitaleX™ Enrichment

BS12 CLSI CMPH2 MCM9

United States and Canada

Cat. No. 221240 Prepared Plates (150 × 15 mm-style) – Pkg. of 8*

Japan

Cat. No. 252044 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

GC-Lect™ Agar

Intended Use

BBL™ GC-Lect™ Agar is a selective plated medium providing enhanced growth and recovery of *Neisseria gonorrhoeae* and better inhibition of contaminating bacteria and fungi, including *Capnocytophaga* species in oropharyngeal specimens.

Summary and Explanation

A succession of media have been developed for the isolation of the pathogenic *Neisseria* from specimens containing mixed flora (Thayer-Martin Selective Agar, Modified Thayer-Martin [MTM] Agar, Martin-Lewis Agar).[1-3] Each provides greater inhibition of contaminating organisms than the preceding formulation but each is, to varying degrees, inhibitory to certain strains that it is designed to recover.[4,5]

BD Diagnostics developed **BBL™** GC II Agar Base as an improved base for Chocolate II Agar, which is utilized in these selective media. The superior growth-promotion achieved for pathogenic *Neisseria* also enabled growth of stains of *Capnocytophaga* on the selective medium when inoculated with oropharyngeal specimens.[6]

GC-Lect Agar was developed and patented by BD Diagnostics to provide the additional inhibition required to prevent overgrowth of the pathogenic *Neisseria* in specimens containing *Capnocytophaga* species and other strains resistant to the inhibitors in MTM Agar; i.e., vancomycin-resistant contaminants, including certain strains of *Staphylococcus epidermidis*. As with MTM, *N. lactamica*, which is resistant to colistin, is not inhibited by **GC-Lect** Agar.

GC-Lect Agar contains a decreased concentration of vancomycin for improved recovery of *N. gonorrhoeae* strains that are sensitive to this antibiotic.

The JEMBEC™* system consists of **GC-Lect** Agar in a JEMBEC (John E. Martin Biological Environmental Chamber)-style plate, a carbon dioxide tablet and resealable plastic bag.[7]

A 100 mm dish (with pill pocket) is also available with **GC-Lect** Agar as the medium.

**JEMBEC is a trademark of Miles Scientific.*

Principles of the Procedure

BBL™ GC-Lect™ Agar is based on **BBL** Chocolate II Agar that contains the improved GC II Agar Base, achieved through careful selection and pretesting of raw materials, bovine hemoglobin and **BBL™ IsoVitaleX™** Enrichment. The GC II Agar Base contains nitrogenous nutrients in the form of casein and meat peptones,

phosphate buffer to maintain pH and corn starch, which neutralizes toxic fatty acids that may be present in the agar. X (hemin) and V (nicotinamide adenine dinucleotide) factors are provided by hemoglobin and **IsoVitaleX** Enrichment. **IsoVitaleX** Enrichment also provides vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors that improve the growth of pathogenic *Neisseria*.[8] In addition, **GC-Lect** Agar permits the growth of some vancomycin-sensitive gonococcal strains which are inhibited on standard MTM Agar.[9]

To improve the selectivity of **GC-Lect** Agar, a combination of five antimicrobial agents was developed to inhibit gram positive bacteria, including vancomycin-resistant *S. epidermidis*, gram-negative species, including *Proteus* and *Capnocytophaga*, as well as fungi, including *Candida albicans*.[9]

In the JEMBEC system, a tablet consisting of a mixture of citric acid and sodium bicarbonate is placed in a well within the plate and is activated by the moisture (humidity) produced by the culture medium within the sealed plastic bag. The CO_2 levels generated are sufficient for the growth of *Neisseria gonorrhoeae*.[7]

Procedure

Streak the specimen as soon as possible after it is received in the laboratory. If material is being cultured directly from a swab, proceed as follows.[10]

1. Roll swab directly on the medium in a large "Z" to provide adequate exposure of swab to the medium for transfer of organisms.

JEMBEC™ System
Neisseria gonorrhoeae
ATCC™ 43069

2. Cross-streak the "Z" pattern with sterile wire loop, preferably in the clinic. If not done previously, cross-streaking should be done in the laboratory.
3. Place the culture as soon as possible in an aerobic environment enriched with carbon dioxide.
   With the JEMBEC System: With sterile forceps remove a CO_2-generating tablet from its foil wrapper and place it in the specially designed well in the plate. Place inoculated plates in the polyethylene bag provided (one plate per bag). DO NOT ADD WATER TO THE TABLET. Seal the bag by pressing down on the "zipper" at the end of the bag with fingers and slide along to the opposite end. Be sure that the bag is sealed completely. After the bag is sealed, incubate in an inverted position (agar side up) at 35°C for 18-48 hours.[11]
   To transport the culture after incubation, place the sealed JEMBEC system in a suitable mailing or shipping container. Care should be taken to protect the culture from extreme heat or cold and to ensure delivery to the testing laboratory as soon as possible.
4. Incubate at 35 ± 2°C and examine after overnight incubation and again after approximately 48 hours.
5. Subculture for identification of *N. gonorrhoeae* should be made within 18-24 hours. If shipped after incubation, colonies should be subcultured before performing biochemical identification tests in order to ensure that adequate viability is achieved.

Expected Results

After a minimum of 18 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Some strains may require up to 72 hours of incubation before visible colonies appear.

Neisseria gonorrhoeae appears as small, grayish-white to colorless mucoid colonies. *N. meningitidis* forms a colony similar to *N. gonorrhoeae*, but larger and bluish-gray.

A presumptive identification may be made by performing a Gram stain and an oxidase test.[12] Biochemical tests and other identification procedures should be performed to confirm findings.

Limitation of the Procedure

While the JEMBEC system is an improvement over previous transport systems, optimum recovery of *N. gonorrhoeae* will be obtained by direct inoculation of media with the specimen and immediate incubation in a CO_2-enriched atmosphere at 35°C.

Specific Performance Characteristics[10]

In a clinical study with 500 specimens, visible growth of *N. gonorrhoeae* occurred within 24 hours in 72% of the positive cultures on **GC-Lect** Agar, compared with only 52% on the reference medium, MTM Agar. A total of 50 positive cultures were obtained with **GC-Lect** Agar, compared with 49 obtained with MTM. The selectivity of **GC-Lect** Agar was superior, with only 19 cultures producing growth of normal flora, compared with 78 cultures on MTM after 24 hours of incubation. The selectivity was especially improved on

GC-Lect Agar with regard to yeasts (2 versus 30 cultures) and gram-positive cocci (5 versus 31 cultures).

References

1. Thayer and Martin, 1966. Pub. Health Rep. *81*:559.
2. Martin, Armstrong and Smith. 1974. Appl. Microbiol. 27:802.
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12. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ GC-Lect™ Agar

United States and Canada

Cat. No.	297715	Prepared Plates – Pkg. of 20*
	297928	Prepared Plates – Ctn. of 100*
	298243	Prepared 100 mm Dish (Pill-pocket) – Ctn. of 100*
	221995	JEMBEC Plate – Pkg. of 10*

Europe

Cat. No.	254554	Prepared Plates – Pkg. of 20*
	254555	Prepared Plates – Ctn. of 120*

**Store at 2-8°C.*

GN Broth • GN Broth, Hajna

Intended Use

GN Broth is used for the selective enrichment of *Salmonella* and *Shigella*.

Summary and Explanation

GN (Gram Negative) Broth was developed by Hajna as an enrichment medium for the recovery of *Salmonella* and *Shigella* from clinical and nonclinical specimens.[1,2] Croft and Miller succeeded in isolating more *Shigella* strains by use of this medium, rather than by direct streaking.[3] Taylor and Schelhart reported that GN Broth enhanced the isolation of enteric pathogens, producing a 53% increase in *Shigella* and a 36% increase in *Salmonella* as compared to direct streaking.[4] In another study, Taylor and Schelhart showed that GN Broth was superior to selenite enrichment media for the isolation of *Shigella*.[5]

GN Broth currently is recommended for use in the microbiological examination of foods.[6]

Principles of the Procedure

Peptones provide amino acids and other nitrogenous substances to support bacterial growth. Mannitol and dextrose are sources of energy. Mannitol is provided in a higher concentration than dextrose to enhance the growth of mannitol-fermenting species, such as *Salmonella* and *Shigella*, and limit the growth of *Proteus* and other dextrose-fermenting bacteria. Phosphate

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ GN Broth, Hajna

Dehydrated Appearance:	Off-white to light tan, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in purified water. Solution is light amber, clear to slightly opalescent.
Prepared Appearance:	Light amber, clear to slightly opalescent.
Reaction of 3.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ GN Broth, Hajna

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	19433	10^3-2×10^3	None to poor
Escherichia coli	25922	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good
Shigella flexneri	12022	10^2-10^3	Good

Identity Specifications

BBL™ GN Broth

Dehydrated Appearance:	Fine, dry, homogeneous, free of extraneous material.
Solution:	3.9% solution, soluble in purified water. Solution is pale to medium, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Pale to medium, tan to yellow, clear to slightly hazy.
Reaction of 3.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

BBL™ GN Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C; subculture to MacConkey II Agar after 6 hours and again after 18-24 hours of incubation. Incubate subculture plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good
Shigella sonnei	9290	10^2-10^3	Good

buffers are incorporated to maintain the pH of the medium. Sodium citrate and sodium desoxycholate are added to inhibit gram-positive and some gram-negative bacteria.

Proteus, *Pseudomonas* and coliforms do not overgrow *Salmonella* and *Shigella* in GN Broth during the first 6 hours of incubation.

Formulae

Difco™ GN Broth, Hajna

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	12.0	g
Proteose Peptone No. 3	8.0	g
Dextrose	1.0	g
D-Mannitol	2.0	g
Sodium Citrate	5.0	g
Sodium Desoxycholate	0.5	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.5	g
Sodium Chloride	5.0	g

BBL™ GN Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Dextrose	1.0	g
D-Mannitol	2.0	g
Sodium Citrate	5.0	g
Sodium Desoxycholate	0.5	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.5	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 39 g of the powder in 1 L of purified water. Mix thoroughly.
2. Dispense and autoclave at 121°C for 15 minutes.
3. Alternatively, the broth may be steamed for 30 minutes at 100°C.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the broth as soon as possible after the specimen arrives at the laboratory. Swab specimens may be inserted directly into the broth. For stool specimens, use 1 g of feces or 1 mL of liquid stool per tube. Consult appropriate references for information about the processing and inoculation of other clinical specimens or food samples.[6-9]

Incubate the tubes with loosened caps at 35 ± 2°C and subculture onto selective and differential media after 6-8 hours of incubation and again after 18-24 hours of incubation.[10]

Expected Results

Growth in broth media is indicated by turbidity compared to an uninoculated control. Subculture onto appropriate selective and differential media to isolate pathogens for identification.

Limitation of the Procedure

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult references for detailed information and recommended procedures.[6-9]

References

1. Hajna. 1955. Public Health Lab. *13*:59.
2. Hajna. 1955. Public Health Lab. *13*:83.
3. Croft and Miller. 1956. Am. J. Clin. Pathol. *26*:411.
4. Taylor and Schelhart. 1967. Am. J. Clin. Pathol. *48*:356.
5. Taylor and Schelhart. 1968. Appl. Microbiol. *16*:1383.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
8. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
9. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
10. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ GN Broth, Hajna

BS12 CCAM CMPH2 COMPF MCM9

Cat. No. 248610 Dehydrated – 500 g

BBL™ GN Broth

BS12 CCAM CMPH2 COMPF MCM9

Cat. No.	211279	Dehydrated – 500 g
	221729	Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
	221730	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*

**Store at 2-8°C.*

Gelatin

Intended Use

Gelatin is used in preparing microbiological culture media.

Summary and Explanation

Gelatin is a protein of uniform molecular constitution derived chiefly by the hydrolysis of collagen.[1] Collagens are a class of albuminoids found abundantly in bones, skin, tendon, cartilage and similar animal tissues.[1]

Koch[1] introduced gelatin into bacteriology when he invented the gelatin tube method in 1875 and the plate method in 1881. This innovation, a solid culture method, became the foundation for investigation of the propagation of bacteria.[1] However, gelatin-based media were soon replaced by media containing agar as the solidifying agent.

Gelatin is used in culture media for determining gelatinolysis (elaboration of gelatinases) by bacteria. Levine and Carpenter[2] and Levine and Shaw[3] employed gelatin media in their studies of gelatin liquefaction. Garner and Tillett[4] used culture media prepared with gelatin to study the fibrinolytic activity of hemolytic streptococci.

User Quality Control

Identity Specifications

Difco™ Gelatin

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	12% solution, soluble in purified water upon slight heating in a 50-55°C water bath. Solution is light amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Gel:	Very light amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 2% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Gelatin

Prepare a 12% Gelatin solution in 0.8% Nutrient Broth. Dispense into tubes and autoclave. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 18-48 hours or for up to 2 weeks for the gelatinase test. To read gelatinase, refrigerate until well-chilled and compare to uninoculated tubes. Tubes positive for gelatinase will remain liquid.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GELATINASE
Bacillus subtilis	6633	10^2-10^3	Good	+
Clostridium sporogenes	11437	10^2-10^3	Good	+
Escherichia coli	25922	10^2-10^3	Good	–

Uninoculated Tube

Bacillus subtilis ATCC™ 6633

Gelatin is a high grade gelatin in granular form which may be used as a solidifying agent or may be incorporated into culture media for various uses. Gelatin is used in Nutrient Gelatin, Motility GI Medium, Stock Culture Agar and Dextrose Starch Agar. A 0.4% gelatin medium is used in the presumptive differentiation of *Nocardia brasiliensis* from *N. asteroides* (see Nocardia Differentiation Media). Media containing gelatin are specified in standard methods[5,6] for multiple applications.

Principles of the Procedure

The melting point of a 12% concentration of gelatin is between 28 and 30°C, which allows it to be used as a solidifying agent. Certain microorganisms elaborate gelatinolytic enzymes (gelatinases) which hydrolyze gelatin, causing liquefaction of a solidified medium or preventing the gelation of a medium containing gelatin. Gelatin is also used as a source of nitrogen and amino acids.

Procedure

See appropriate references for specific procedures using gelatin.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Gershenfeld and Tice. 1941. J. Bacteriol. *41*:645.
2. Levine and Carpenter. 1923. J. Bacteriol. *8*:297.
3. Levine and Shaw. 1924. J. Bacteriol. *9*:225.
4. Garner and Tillett. 1934. J. Exp. Med. *60*:255.
5. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
6. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.

Availability

Difco™ Gelatin

Cat. No.	214340	Dehydrated – 500 g
	214320	Dehydrated – 10 kg

Gelysate™ Peptone

Intended Use

Gelysate Peptone is used for cultures requiring low carbohydrates, cystine and tryptophan levels in cell culture and bacterial fermentation.

Summary and Explanation

Gelatin hydrolysate is high in proline residues.[1] **Gelysate** Peptone is deficient in carbohydrates and is characterized by low cystine, methionine and tryptophan content. When used alone as a basic nutrient, it is suitable for preparing media for organisms not particularly fastidious in their nutritional requirements.

Consult standard methods manuals for media formulations containing **Gelysate** Peptone.[2,3]

User Quality Control

Identity Specifications

BBL™ Gelysate™ Peptone

Dehydrated Appearance:	Tan, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water. Solution is clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 6.5-7.5

Cultural Response
Biochemical Reactions

BBL™ Gelysate™ Peptone

Prepare a sterile solution of **Gelysate** Peptone as directed below. Adjust final pH to 7.2-7.4. Inoclate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	29552	~10^7	Negative
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Negative
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Citrobacter freundii*	8454	0.1 mL, undiluted	Positive

Growth Response

BBL™ Gelysate™ Peptone

Prepare a sterile solution of 10 g of **Gelysate** Peptone, 2.5 g of sodium chloride and 6.5 g of agar in 500 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 2-3 days (incubate *S. pyogenes* with 3-5% CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	10^3-10^4	Good
Pseudomonas aeruginosa	27853	10^3-10^4	Good
Staphylococcus aureus	6538P	10^3-10^4	Good
Streptococcus pyogenes	49117	10^4-10^5	Good

Principles of the Procedure

Gelysate Peptone is a pancreatic digest of gelatin. Gelatin is extracted from collagen, which is the fibrous protein in bone, cartilage and connective tissue. **Gelysate** Peptone provides nitrogen, amino acids and vitamins in microbiological culture media.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Gelysate** Peptone in the formula of the medium being prepared. Add product as required.

Procedure

See appropriate references for specific procedures using **Gelysate** Peptone.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Bridson and Brecker. 1970. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York, N.Y.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
3. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

BBL™ Gelysate™ Peptone

BAM USDA

Cat. No. 211870 Dehydrated – 454 g

Giolitti-Cantoni Broth Base

Intended Use

Giolitti-Cantoni Broth Base is used for enriching *Staphylococcus aureus* from foods during isolation procedures.

Summary and Explanation

Giolitti and Cantoni[1] described a broth medium with added potassium tellurite and a test procedure for enriching small numbers of staphylococci in foods. Mossel et al.[2] recommended Giolitti-Cantoni Broth for detecting *Staphylococcus aureus* in dried milk and other infant foods where the organism should be absent from 1 g of test material.

The International Organization for Standardization (ISO) and American Public Health Association recommend using Giolitti-Cantoni Broth in a MPN procedure for detecting *S. aureus*.[3,4]

Principles of the Procedure

Giolitti-Cantoni Broth Base contains peptone and beef extract as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. D-Mannitol is the carbohydrate source. Sodium pyruvate stimulates growth of staphylococci. Lithium chloride inhibits gram-negative bacilli. Added Tellurite Solution 1% supplies potassium tellurite, which in combination with glycine, inhibits gram-positive bacteria other than staphylococci. Tween™* 80 acts as an emulsifier.

**Tween is a trademark of ICI Americas Inc.*

Formula

Difco™ Giolitti-Cantoni Broth Base

Approximate Formula* Per Liter

Tryptone	10.0	g
Beef Extract	5.0	g
Yeast Extract	5.0	g
D-Mannitol	20.0	g
Sodium Chloride	5.0	g
Lithium Chloride	5.0	g
Glycine	1.2	g
Sodium Pyruvate	3.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 54.2 g of the powder in 1 L of purified water.
2. Add 1.0 g Tween 80.
3. Warm slightly to completely dissolve the powder.
4. Dispense 19 mL amounts into 20 × 200 mm tubes.
5. Autoclave at 121°C for 15 minutes. Cool to 15-30°C.
6. Aseptically add 1.05 mL Tellurite Solution 1% per tube; 0.105 mL when testing meat products (or quality control organisms). Mix thoroughly.
7. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[3,4]

Consult appropriate references for details on test methods using Giolitti-Cantoni Broth Base.[3,4]

Expected Results

Read tubes for blackening of the medium (a positive reaction) or no blackening (a negative reaction). If blackening occurs, subculture to Baird-Parker Agar to confirm the isolation of *S. aureus*.

User Quality Control

Identity Specifications

Difco™ Giolitti-Cantoni Broth Base

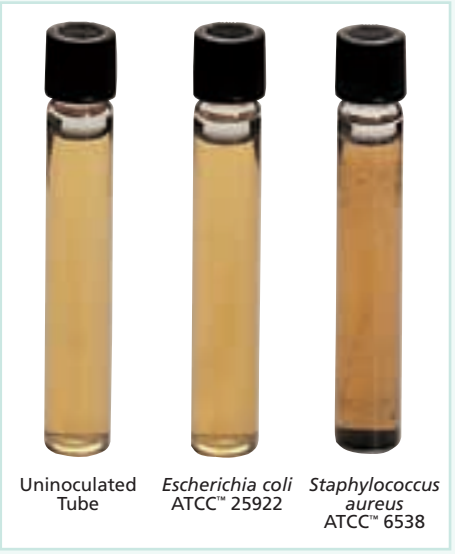
Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.42% solution, soluble in purified water upon warming. Solution is medium amber, clear.
Prepared Appearance:	Medium amber, clear.
Reaction of 5.42% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Giolitti-Cantoni Broth Base

Prepare the medium per label directions. Inoculate (overlaying with sterile paraffin wax or mineral oil) and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
Escherichia coli	25922	10^3-2×10^3	Inhibition	No blackening
Micrococcus luteus	10240	10^3-2×10^3	Inhibition	No blackening
Staphylococcus aureus	6538	10^2-10^3	Good	Black
Staphylococcus aureus	25923	10^2-10^3	Good	Black



Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Staphylococcus aureus* ATCC™ 6538

References

1. Giolitti and Cantoni. 1966. J. Appl. Bacteriol. 29:395.
2. Mossel, Harrewijn and Elzebroek. 1973. UNICEF.
3. International Organization for Standardization. 2003. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci – Part 3: Detection and MPN technique for low numbers. ISO 6888-3, 2003-03-15. ISO, Geneva, Switzerland.
4. Downes and Ito (ed.). 2001. Compendium of methods for the microbiogical examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Giolitti-Cantoni Broth Base

COMPF ISO

Cat. No. 218091 Dehydrated – 500 g

BBL™ Tellurite Solution 1%

Cat. No. 211917 Tube – 20 mL

Difco™ Tween™ 80

Cat. No. 231181 Bottle – 100 g

M-Green Yeast and Mold Broth

Intended Use

M-Green Yeast and Mold Broth is used for the detection of fungi in the routine analysis of beverages.

Summary and Explanation

M-Green Yeast and Mold Broth is an improved modification of the liquid medium, M-Yeast and Mold Broth, which was developed to improve the efficiency of detection and enumeration of fungi in sugar and other materials by the membrane filter method. The revised formula contains the indicator dye, bromcresol green. It is a relatively more complex formula than many of the other media exclusively used for the recovery of yeasts and molds.

Principles of the Procedure

The formulation is rich in nutrients provided by peptones, yeast extract and dextrose, but bacterial growth is inhibited by the acid pH. Diastase is a mixture of amylolytic (starch-hydrolyzing) enzymes. The bromcresol green indicator facilitates the visualization and counting of fungal colonies. The colonies are green due to the diffusion of bromcresol green into the colonies (alkaline reaction). End products from the colonies diffuse into the medium, further reducing the pH and causing the dye to turn yellow (acid reaction).

Formula

BBL™ M-Green Yeast and Mold Broth

Approximate Formula* Per Liter

Yeast Extract	9.0	g
Dextrose (anhydrous)	50.0	g
Pancreatic Digest of Casein	5.0	g
Peptic Digest of Animal Tissue	5.0	g
Magnesium Sulfate	2.1	g
Potassium Phosphate	2.0	g
Diastase	0.05	g
Thiamine	0.05	g
Bromcresol Green	26.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 7.3 g of the powder in 100 mL of purified water. Mix thoroughly.
2. Warm slightly if necessary to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

BBL™ M-Green Yeast and Mold Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain a large number of minute to small, dark green and/or white specks.
Solution:	7.3% solution, soluble in purified water. Solution is medium, green (with or without a tint of yellow) to blue-green, clear to moderately hazy.
Prepared Appearance:	Medium, green to blue-green, clear to moderately hazy.
Reaction of 7.3% Solution at 25°C:	pH 4.6 ± 0.2

Cultural Response

BBL™ M-Green Yeast and Mold Broth

Prepare the medium per label directions. Inoculate using the membrane filter technique and incubate at 30-35°C for 2 days (up to 5 days, if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10^2-3×10^2	Good
Candida tropicalis	1369	30-300	Good
Penicillium roquefortii	10110	10^2-3×10^2	Good
Saccharomyces cerevisiae	9763	30-300	Good

Procedure

1. Saturate a sterile membrane filter pad in a sterile Petri dish with 2.0-2.5 mL of M-Green Yeast and Mold Broth.
2. Roll a membrane filter, which has been used to filter the test sample, onto the surface of the moistened pad so as to avoid the trapping of air bubbles between the filter and the pad.
3. Incubate the plates at 30-35°C for 48 hours and up to 5 days in an aerobic atmosphere with increased humidity.

Expected Results

After incubation, colonies appearing on the filter surface can be counted. Mold colonies generally appear green and filamentous, whereas yeast colonies are green and opaque.

Availability

BBL™ M-Green Yeast and Mold Broth

COMPF

Cat. No.	211286	Dehydrated – 100 g*
	211287	Dehydrated – 500 g*

**Store at 2-8°C.*

Group A Selective Strep Agar with 5% Sheep Blood (ssA™)

Intended Use

Group A Selective Strep Agar with 5% Sheep Blood (**ssA**™) is recommended as a primary selective plating medium for the isolation of group A streptococci (*S. pyogenes*) from throat cultures and other specimens in which the presence of *S. pyogenes* is suspected. Group B streptococci will also grow on this medium; most other streptococci, neisseriae, staphylococci and gram-negative bacteria are inhibited. The medium is designed for use is conjunction with **Taxo**™ A (bacitracin, 0.04 unit) discs for presumptive identification of *S. pyogenes*.

Summary and Explanation

Infection with Lancefield group A streptococci (*S. pyogenes*) may produce serious sequelae such as rheumatic fever and acute glomerulonephritis. Therefore, early detection and identification are important.

Because of the overgrowth of normal flora present in throat culture specimens inoculated onto routine blood agar plates, selective ingredients have been added to sheep blood agar to enhance the detection of group A streptococci.

Evaluation of various antimicrobial agents resulted in a combination with improved selectivity over other selective media tested. This medium (**ssA**) allows presumptive identification of group A streptococci, based on bacitracin susceptibility and beta hemolysis, within 24 hours after inoculation with the specimen when the medium is incubated in a CO_2-enriched atmosphere.[1]

Principles of the Procedure

The base medium, modified **Trypticase** Soy Agar (TSA II), provides a combination of casein and soy peptones that supply organic nitrogen. The sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood provides proper hemolytic reactions of streptococci. In addition, growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited. The incorporation of a unique combination of selective ingredients provides improved suppression of normal throat flora for improved recovery of *S. pyogenes*.[1,2]

Beta-hemolytic streptococci which show a zone of inhibition around a bacitracin (0.04 unit) disc may be presumptively identified as group A streptococci.[3]

Procedure

As soon as possible, inoculate the specimen onto a Group A Selective Strep Agar with 5% Sheep Blood (**ssA**) plate by firmly rolling the swab over a third of the agar surface. Streak the remainder of the plate with a sterilized or sterile disposable inoculating loop to obtain isolated colonies. After streaking, stab the agar two or three times in the area of heaviest inoculation.

Place **Taxo** A disc directly on the swabbed portion of the plate; i.e., where the swabbed area is intersected by the area of initial loop streaking. It is recommended that a routine blood agar plate, such as **Trypticase** Soy Agar with 5% Sheep Blood (TSA II) also be inoculated to assure the recovery of microorganisms that may be inhibited on the selective medium.

Incubate inoculated plates at 35 ± 2°C in an atmosphere enriched with carbon dioxide. If plates are incubated without carbon dioxide, the beta-hemolytic zones and colony size will be smaller and fewer colonies may be apparent.

Expected Results

After 18-24 hours of incubation in an atmosphere enriched with carbon dioxide, group A streptococci (*S. pyogenes*) will appear as translucent or opaque, white to gray, small (1- 2 mm) colonies surrounded by a zone of beta hemolysis. A decrease in size as compared to a nonselective control is typical. Pinpoint or very small colonies of alpha-, nonhemolytic or other beta-hemolytic streptococci may grow in small numbers, but they should not interfere with the recovery of group A streptococci or interpretation of the results. *Neisseria* species, viridans streptococci, staphylococci, gram-negative rods and most beta-hemolytic streptococci other than groups A and B are inhibited. Bacitracin susceptibility may be used to differentiate group A streptococci from group B. Fair to heavy growth of beta-hemolytic colonies demonstrating a zone of inhibition around the **Taxo** A disc on this medium may be

Streptococcus pyogenes
ATCC™ 19615

presumptively reported as *S. pyogenes*. A PYR (pyroglutamic acid) test may also be performed. It is more specific than the bacitracin test for this purpose.[4] Gram stains should be made and examined.

A serological grouping test procedure may be performed if sufficient well-isolated beta-hemolytic colonies are present.

References

1. Evans and O'Neill, 1984. Abstr. C-136. Abstr. 84th Annu. Meet. Am. Soc. for Microbiol. 1984.
2. Carlson, Merz, Hansen, Ruth and Moore. 1985. J. Clin. Microbiol. *21*:307.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Facklam and Washington. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Group A Selective Strep Agar with 5% Sheep Blood (ssA™)

BS12 MCM9

United States and Canada

Cat. No.	221779	Prepared Plates – Pkg. of 20*
	221780	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254050	Prepared Plates – Pkg. of 20*

Japan

Cat. No.	212498	Prepared Plates – Pkg. of 20*

BBL™ Group A Selective Strep Agar with 5% Sheep Blood (ssA™)//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No.	221783	Prepared Bi-Plate Dishes – Pkg. of 20*

**Store at 2-8°C.*

HBT Bilayer Medium

Intended Use

HBT (Human Blood Tween™*) Bilayer Medium is a selective and differential medium used in the primary isolation and presumptive identification of *Gardnerella vaginalis* from clinical specimens.

**Tween is a trademark of ICI Americas, Inc.*

Summary and Explanation

HBT Bilayer Medium was described in 1982 by Totten et al. for the selective isolation and detection of *G. vaginalis* from clinical specimens.[1] The basal medium consists of Columbia Agar supplemented with selective agents and Tween (polysorbate) 80. The top layer is made by adding human blood to the basal medium. Colonies of *G. vaginalis* may be differentiated by a diffuse, beta-hemolytic reaction they produce in the presence of human blood.[2-5] Totten et al. reported that the rate of isolation of *G. vaginalis* was better on HBT Bilayer Medium than on a single layer human blood medium because the characteristic beta-hemolytic reaction used in differentiating the bacterium was more apparent.[1]

Principles of the Procedure

Columbia Agar supplies the nutrients necessary to support bacterial growth. Enzymatic digests of casein, meat and beef extract provide amino acids and other complex nitrogenous substances. Yeast extract primarily supplies the B-complex vitamins. Corn starch is incorporated to neutralize fatty acids that may be toxic to *G. vaginalis*. Sodium chloride maintains the osmotic equilibrium.

Colistin, nalidixic acid (CNA) and amphotericin B are added to facilitate the selective recovery of *G. vaginalis* from clinical specimens. Colistin and nalidixic acid inhibit most gram-negative organisms. Amphotericin B is active against yeasts and filamentous fungi.

A thin layer of the medium with human blood enhances the detection of the characteristic diffuse beta hemolysis of *G. vaginalis*.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 35 ± 2°C in a CO_2-enriched atmosphere for 24-48 hours.

Expected Results

G. vaginalis produces small, white colonies surrounded by a beta-hemolytic zone with a diffuse edge.

References

1. Totten, Amsel, Hale, Piot and Holmes. 1982. J. Clin. Microbiol. *15*:141.
2. Greenwood and Pickett. 1979. J. Clin. Microbiol. *9*:200.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
5. Funke and Bernard. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ HBT Bilayer Medium

BS12 CMPH2

Cat. No.	297884	Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

HC Agar Base

Intended Use

HC Agar Base, when supplemented with Polysorbate 80, is used for enumerating molds in cosmetic products.

Summary and Explanation

Methods for isolating molds from cosmetic products require incubation for 5 to 7 days using traditional agar media.[1] In 1986, Mead and O'Neill[2] described a new medium, HC Agar, for enumerating molds in cosmetic products that decreased incubation time to 3 days at 27.5 ± 0.5°C. HC Agar Base, based on the HC Agar formula of Mead and O'Neill, is supplemented with Polysorbate 80 to prepare HC Agar.

Principles of the Procedure

HC Agar Base contains peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose provides a source of fermentable carbohydrate. Ammonium chloride and magnesium sulfate provide essential ions. Disodium and monopotassium phosphates buffer the pH to near neutrality. Sodium carbonate inactivates low levels of preservatives that are active at a more acidic pH (e.g., benzoic acid). Chloramphenicol inhibits bacteria, including *Pseudomonas aeruginosa* and *Serratia marcescens*, that are potential contaminants of cosmetic products. Polysorbate 80 neutralizes preservatives and sequesters surfactants that may be present in residual amounts from the product sample.[2] Agar is the solidifying agent.

Formula

Difco™ HC Agar Base

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	2.5	g
Proteose Peptone	2.5	g
Yeast Extract	5.0	g
Dextrose	20.0	g
Disodium Phosphate	3.5	g
Monopotassium Phosphate	3.4	g
Ammonium Chloride	1.4	g
Magnesium Sulfate	0.06	g
Chloramphenicol	0.1	g
Sodium Carbonate	1.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 54.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 20 mL of Polysorbate 80.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ HC Agar Base

Dehydrated Appearance:	Very light to light beige, free-flowing, homogeneous.
Solution:	5.45% solution, soluble in purified water upon boiling. Solution is medium to dark amber, slightly opalescent to opalescent, may have a slight precipitate.
Prepared Appearance:	Medium amber with yellow tint, very slightly to slightly opalescent, no significant precipitate.
Reaction of 5.45% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ HC Agar Base

Prepare the medium per label directions. Inoculate and incubate at 27.5 ± 0.5°C for 65-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10^2-10^3	Good
Pseudomonas aeruginosa	10145	10^3-2×10^3	None to poor
Serratia marcescens	13880	10^3-2×10^3	None to poor

Procedure

1. Process each specimen as appropriate for that specimen and inoculate directly onto the surface of the medium.[1] Inoculate duplicate plates.
2. Incubate plates aerobically at 27.5 ± 0.5°C.
3. Examine plates for growth and recovery after 72 hours of incubation.
4. Count mold colonies from duplicate plates and record average count as mold count per gram or milliliter of sample.

Expected Results

Mold cultures should yield good growth and recovery. Bacteria should be inhibited.

Limitation of the Procedure

The 27.5 ± 0.5°C incubation temperature is critical for obtaining statistically significant mold counts after three days using this medium.

References

1. U.S. Food and Drug Administration. 2001. FDA bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. Mead and O'Neill. 1986. J. Soc. Cosmet. Chem. 37:49.

Availability

Difco™ HC Agar Base

Cat. No. 268510 Dehydrated – 500 g

m HPC Agar

Intended Use

m HPC Agar is used for enumerating heterotrophic organisms in treated potable water and other water samples with low counts by membrane filtration.

Summary and Explanation

m HPC Agar was developed by Taylor and Geldreich in 1979 in their pursuit of a suitable standard methods medium to use with the membrane filter procedure.[1] m HPC Agar is also known as m-Heterotrophic Plate Count Agar and previously as membrane filter Standard Plate Count Agar, m-SPC Agar. The formulation was evaluated by many investigators who reported it as a suitable alternative medium for standard plate counts.[2-4] It is recommended for the membrane filter method in the recent editions of *Standard Methods for the Examination of Water and Wastewater*.[5]

The advantages of the membrane filter procedure over the standard plate count method have been described by many investigators.[6-8] The volume of inoculum is limited with both pour and spread plate techniques while the membrane filter method enables the use of large samples, which is desirable for water with low counts.

Principles of the Procedure

Peptone provides nitrogen and carbon as well as other nutrients. The original concentration of 5% gelatin was reduced to 2.5% to avoid problems associated with liquefying gelatin and spreading colonies.

Formula

Difco™ m HPC Agar

Approximate Formula* Per Liter		
Peptone	20.0	g
Gelatin	25.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 6 g of the powder in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 1 mL of glycerol.
4. Autoclave at 121°C for 5 minutes. Cool to 45-50°C.
5. Dispense 5 mL portions into 50 × 9 mm Petri dishes.
6. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Excessive heat may cause breakdown of the gelatin. See "Limitations of the Procedure."

Procedure

Water samples should be collected and handled as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9060.[5]

1. The volume to be filtered will vary with the sample. Select a maximum sample size to give 20-200 CFU per filter.
2. Filter the appropriate volume through a sterile 47 mm, 0.45 µm, gridded membrane filter, under partial vacuum. Rinse funnel with three 20-30 mL portions of sterile dilution water. Place filter on agar in Petri dish.

User Quality Control

Identity Specifications

Difco™ m HPC Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6% solution, soluble in purified water upon boiling. (Add 1% glycerol after boiling.) With glycerol, solution is light amber, slightly opalescent to opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, opalescent, may have a precipitate.
Reaction of 6% Solution with Glycerol at 25°C:	pH 7.1 ± 0.2

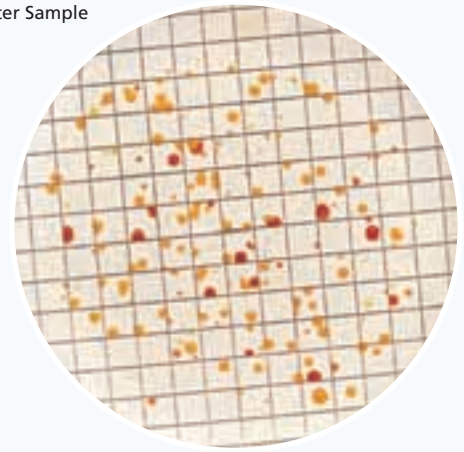
Cultural Response

Difco™ m HPC Agar

Prepare the medium per label directions. Inoculate using the membrane filtration technique. Incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	20-200	Growth
Escherichia coli	25922	20-200	Growth
Pseudomonas aeruginosa	10145	20-200	Growth

Water Sample



3. Place dishes in a close-fitting box or plastic bag containing moistened paper towels.
4. Incubate at 35 ± 0.5°C for 48 hours. Duplicate plates may be incubated at other conditions as desired.

Expected Results

Count all colonies on the membrane when there are 2 or less colonies per square. For 3-10 colonies per square, count 10 squares and obtain average count per square. For 10-20 colonies per square, count 5 squares and obtain average count per square. Multiply average count per square by 100 and divide by the sample volume to give colonies per milliliter. If there are more than 20 colonies per square, record count as > 2,000 divided by the sample volume. Report averaged counts as estimated colony-forming units. Make estimated counts only when there are discrete, separated colonies.[5]

Limitations of the Procedure

1. m HPC Agar is intended for use only with the membrane filter method.
2. m HPC Agar is recommended for testing treated water.
3. Longer incubation times may be necessary to recover slow-growing bacteria.
4. This medium may not be sterile; use with care to avoid contamination.

References

1. Taylor and Geldreich. 1979. J. Am. Water Works Assoc. *71*:402.
2. Means, Hanami, Ridgway and Olson. 1981. J. Am. Water Works Assoc. *73*:585.
3. Nagy and Olson. 1982. Can. J. Microbiol. *28*:667.
4. Haas, Meyer and Paller. 1982. J. Am. Water Works Assoc. *74*:322.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
6. Lechevallier, Seidler and Evans. 1980. Appl. Environ. Microbiol. *40*:922.
7. Stapert, Sokolski and Northam. 1962. Can. J. Microbiol. *8*:809.
8. Saleem and Schlitzer. 1983. Abstr. Q 126, p. 281. Abstr. 83rd Annu. Meet. Am. Soc. Microbiol. 1983.

Availability

Difco™ m HPC Agar

COMPF SMWW

Cat. No.	275220	Dehydrated – 500 g

Difco™ Glycerol

Cat. No.	228210	Bottle – 100 g
	228220	Bottle – 500 g

Haemophilus Isolation Agar with Bacitracin

Intended Use

Haemophilus Isolation Agar with Bacitracin is a primary plating medium used for the selective isolation of *Haemophilus* species.

Summary and Explanation

Members of the genus *Haemophilus* are fastidious microorganisms that require the addition of the growth factors hemin (X factor) and/or nicotinamide adenine dinucleotide (NAD or V factor).[1] To enable the cultivation of *Haemophilus* species, Haemophilus Isolation Agar is formulated with Fildes Enrichment and **BBL™ IsoVitaleX™** Enrichment to supply the essential X and/or V growth factors. Horse blood provides appropriate hemolytic reactions to facilitate the differentiation of *Haemophilus* species.

The antimicrobial agent bacitracin is incorporated to inhibit the growth of bacteria that could mask the presence of *Haemophilus* species. Bacitracin is frequently utilized in enriched media as a selective agent to increase the recovery of *Haemophilus* species from the upper respiratory tract.[2]

Principles of the Procedure

Haemophilus Isolation Agar with Bacitracin consists of Brain Heart Infusion Agar supplemented with Fildes Enrichment, **IsoVitaleX** Enrichment and horse blood. Fildes Enrichment is a peptic digest of sheep blood that supplies both X and V factors.[3,4]

IsoVitaleX Enrichment is a chemically-defined supplement that provides V factor and other nutrients, such as thiamine and cysteine, to stimulate the growth of *Haemophilus* species.[5] The horse blood supplies additional nutrients and enables the detection of hemolytic reactions, which aid in the differentiation and identification of *Haemophilus* species.

The polypeptide antibiotic bacitracin is incorporated into these media to inhibit normal flora, including gram-positive bacteria, such as streptococci, and most species of *Neisseria*.[6]

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 35°C in a moist, CO_2-enriched atmosphere for 24-48 hours to obtain satisfactory growth of *H. influenzae* and most other *Haemophilus* spp. *H. aegyptius* requires a longer incubation period, 2-4 days. *H. ducreyi* may require up to 9 days of incubation, preferably at a temperature of 33°C.[7]

Expected Results

Haemophilus influenzae produces pale gray, smooth, glistening and slightly convex colonies. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

References

1. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Chapin and Doern. 1983. J. Clin. Microbiol. *17*:1163.
3. Fildes. 1920. Br. J. Exper. Pathol. *1*:129.
4. Fildes. 1921. Br. J. Exper. Pathol. *2*:16.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergeys Manual of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Garrod and O'Grady. 1971. Antibiotics and chemotherapy, 3rd ed. Williams & Wilkins, Baltimore, Md.
7. Kilian. 1991. *In* Balow, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Haemophilus Isolation Agar with Bacitracin

Cat. No. 295914 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Haemophilus Test Medium Agar (HTM Agar)

Intended Use

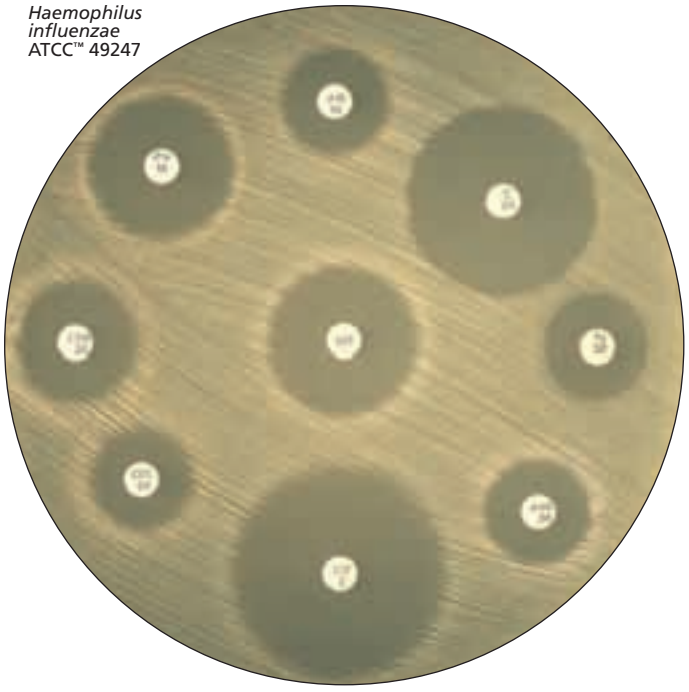
Haemophilus Test Medium Agar (HTM Agar) is intended for use in the antimicrobial disc diffusion susceptibility procedure for *Haemophilus* spp. as described in the Approved Standard M2, published by the Clinical and Laboratory Standards Institute (CLSI).[1]

Summary and Explanation

In 1966, Bauer, Kirby and others developed a standardized procedure for the antimicrobial susceptibility testing of common, rapidly growing bacteria in which Mueller Hinton Agar was selected as the test medium.[2-4] This medium in not satisfactory for fastidious organisms such as some streptococci, gonococci and *Haemophilus* species.

Mueller Hinton Agar supplemented with 1% hemoglobin and 1% **BBL™ IsoVitaleX™** Enrichment (Mueller Hinton Chocolate Agar) was the medium previously recommended for *Haemophilus influenzae*.[5] Extensive studies performed by Jorgensen and colleagues led to the development of Haemophilus Test Medium (HTM).[6,7] This medium is Mueller Hinton agar or broth supplemented with X factor (hemin or hematin), V factor (nicotinamide adenine dinucleotide, NAD) and yeast extract.

Haemophilus influenzae ATCC™ 49247



A major advantage of HTM Agar compared with Mueller Hinton Chocolate Agar is optical clarity, permitting zone diameter measurements from the bottom of the dish as is the standard test procedure for nonfastidious organisms on Mueller Hinton Agar. Furthermore, HTM Agar contains low levels of thymidine and is, therefore, suitable for testing trimethoprim/sulfamethoxazole.

Interpretive criteria for the antimicrobial susceptibility testing of *Haemophilus* are provided with the CLSI document M2.[1] This document should be consulted for further details.

Principles of the Procedure

The Bauer-Kirby procedure is based on the diffusion through an agar gel of antimicrobial substances which are impregnated on paper discs. In contrast to earlier methods, which used discs of high and low antimicrobial concentrations and the presence or absence of inhibition zones for their interpretation, this method employs discs with single concentration of antimicrobial agent and zone diameters are correlated with minimal inhibitory concentrations (MICs).[1-3,8]

In the test procedure, a standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specified amounts of antibiotic or other antimicrobial agents are then placed on the surface of the medium, the plate is incubated and zones of the inhibition around each disc are measured. The determination as to whether the organism is susceptible, resistant or intermediate in its response to the agent is made by comparing zone diameters obtained to those provided with CLSI document M2.[1]

Various factors have been identified as influencing disc diffusion susceptibility tests. These include the medium, agar depth, disc potency, inoculum concentration, pH and beta-lactamase production by test organisms.[1,5,8,9]

Procedure

1. Prepare a Gram stain before starting susceptibility testing to confirm culture purity and to confirm tentative identification of *Haemophilus*.
2. Use several well-isolated colonies taken directly from an overnight (preferably 20-24 hours) Chocolate Agar plate as the source of the inoculum.
3. A rapid β-lactamase test should be utilized for rapid detection of strains that are resistant to penicillin, ampicillin or amoxicillin.

4. Prepare a suspension of the test organism in Mueller Hinton Broth, Mueller Hinton II Broth or 0.9% saline. This suspension should be adjusted to the turbidity of the 0.5 McFarland standard using a photometric device. This suspension will contain approximately 1-4 × 10^8 CFU/mL. Care must be exercised in preparing this suspension because higher inoculum concentrations may lead to false-resistant results with some β-lactam antibiotics, particularly when β-lactamase-producing strains of *H. influenzae* are tested.[1]
5. Alternative methods of inoculum preparation involving devices that permit direct standardization of inocula without adjustment of turbidity, such as the **BBL™ Prompt™** Inoculation System, have been found to be acceptable for routine testing purposes.[10] This system has also been found to be satisfactory for testing *H. influenzae*.[11]

Consult the product literature or the CLSI Approved Standard M2[1] for details on plate inoculation and use of antimicrobial discs.

Expected Results

1. Examine the plates after 16-18 hours of incubation. A confluent "lawn" of growth should be obtained. If only isolated colonies grow, the inoculum was too light and the rest should be repeated.
2. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using calipers, a ruler, or a template prepared for this purpose. The measuring device is held on the back of the plate, which is held over a black, non-reflecting background and illuminated from above. The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies which can be detected with difficulty near the edge of the obvious zone of the inhibition.
3. Consult the product literature or the CLSI Approved Standard M2[1] for details on interpretation of results.

References

1. Clinical and Laboratory Standards Institute. 2006. Approved standard: M2-A9. Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.
2. Bauer, Kirby, Sherris and Turck. 1966. Am. J. Clin. Pathol. *45*:493.
3. Ryan, Schoenknecht, and Kirby. 1970. Hospital Practice *5*:91.
4. Barry, Garcia, and Thrupp. 1970. Am. J. Clin. Pathol. *53*:149.
5. Neumann, Sahm, Thornsberry and McGowan. 1991. Cumitech 6A, New developments in antimicrobial agent susceptibility testing, A practical guide. Coord. ed., McGowan. American Society for Microbiology, Washington, D.C.
6. Jorgensen, Redding, Maher and Howell. 1987. J. Clin. Microbiol. *25*:2105-2113.
7. Jorgensen, Howell and Maher. J. Clin. Microbiol. *28*:985-988.
8. Ericsson and Sherris. 1971. Acta. Pathol. Microbiol. Scand. Sect. B, Suppl. *217*:1.
9. Jorgensen, Turnidge and Washington. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
10. Baker, Thornsberry and Hawkinson. 1983. J. Clin. Microbiol. *17*:450.
11. Marsik, Evans, Fowler and Thompson. 1989. Abstr. C-67, p. 404. Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989.

Availability

BBL™ Haemophilus Test Medium Agar (HTM Agar)

BS12 CLSI CMPH2

United States and Canada

Cat. No.	221992	Prepared Plates – Pkg. of 10*
	221954	Prepared Plates (150 × 15 mm-style) – Pkg. of 8*

Europe

Cat. No.	254058	Prepared Plates – Pkg. of 20*

Japan

Cat. No.	251992	Prepared Plates – Pkg. of 10*
	252037	Prepared Plates – Pkg. of 20*
	251954	Prepared Plates (150 × 15 mm-style) – Pkg. of 8*

**Store at 2-8°C.*

Heart Infusion Agar

Intended Use

Heart Infusion Agar is a general-purpose medium used in the cultivation of a wide range of microorganisms from a variety of clinical and nonclinical specimens.

Summary and Explanation

Meat infusions provided one of the earliest means of culturing microorganisms, and infusion-based media are still widely used. Huntoon demonstrated that pathogenic organisms could be grown on infusion agar without supplements.[1] In plates, Heart Infusion Agar can be used for primary isolation of organisms from mixed cultures; in agar slant form, it is used primarily for organism cultivation and maintenance rather than for isolation from mixed cultures.

Heart infusion media are specified for the isolation of *Vibrio cholerae* and *Vibrio* species.[2,3] Several modifications of heart infusion media have been described.[4] The **BBL™** prepared medium provided in tubed slants contains yeast extract and a reduced peptone content.

Principles of the Procedure

Heart Infusion Agar derives its nutrients from heart muscle infusion and peptone, which supply nitrogenous and carbonaceous compounds, sulfur, vitamins and trace ingredients. Sodium chloride maintains osmotic equilibrium. Agar is the solidifying agent. The addition of 5% sheep blood provides additional growth factors and is used to determine hemolytic reactions.

Formula

Difco™ Heart Infusion Agar

Approximate Formula* Per Liter		
Beef Heart, Infusion from 500 g	10.0	g
Tryptose	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Heart Infusion Agar

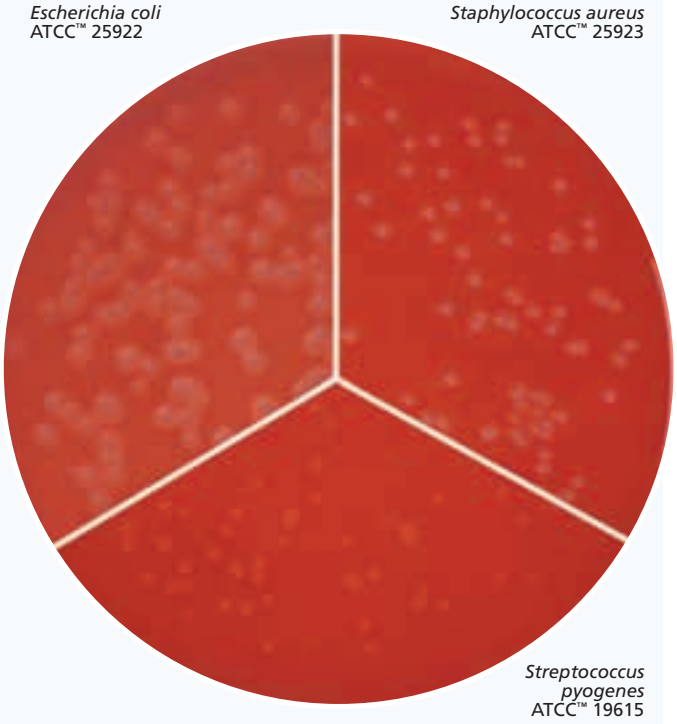
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Plain – Light to medium amber, slightly opalescent. With 5% sheep blood – Cherry red, opaque.
Reaction of 4% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Heart Infusion Agar

Prepare the medium per label directions without (plain) and with 5% sheep blood (SB). Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH 5% SB	HEMOLYSIS
Escherichia coli	25922	10^2-10^3	Good	Good	Beta
Staphylococcus aureus	25923	10^2-10^3	Good	Good	Beta
Streptococcus pneumoniae	6305	10^2-10^3	Fair	Good	Alpha
Streptococcus pyogenes	19615	10^2-10^3	Fair	Good	Beta



Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens.

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2. Incubate plates at 35 ± 2°C for 18-48 hours.

Using a sterile inoculating loop or needle, pick several isolated colonies from the primary isolation plate and streak the surface of a slant of Heart Infusion Agar. Incubate the tubes under appropriate conditions at 35°C.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Huntoon. 1918. J. Inf. Dis. 23:169.
2. U.S. Food and Drug Administration. 2001. FDA bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
3. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. Atlas. 1997. Handbook of microbiological media, 2nd ed. CRC Press, Inc., Boca Raton, Fla.

Availability

Difco™ Heart Infusion Agar

BAM CCAM COMPF

Cat. No.	244400	Dehydrated – 500 g
	244100	Dehydrated – 2 kg
	211839	Dehydrated – 10 kg

BBL™ Heart Infusion Agar

Cat. No.	297336	Tubed Slants – Pkg. of 10

BBL™ Heart Infusion Agar with 5% Sheep Blood

Europe

Cat. No.	257026	Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Bacto™ Heart Infusion Broth

Intended Use

Bacto™ Heart Infusion Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

Heart Infusion Broth (HIB) is a nonselective general-purpose medium used for the isolation of nutritionally fastidious microorganisms. One of the first media used for the cultivation of bacteria was a liquid medium containing an infusion of meat. Huntoon[1] using fresh beef heart and **Bacto** Peptone, prepared a "hormone" broth to retain growth promoting substances. Highly pathogenic organisms, such as meningococci and pneumococci, could be grown on infusion medium without enrichments.[1] The formula for HIB contains tryptose, which is better suited to the nutritional requirements of pathogenic bacteria than **Bacto** Peptone.

Heart infusion media are specified for the isolation of *Vibrio cholerae* and *Vibrio* species.[2,3] HIB may be used as the base in carbohydrate fermentation tests.[4]

Several modifications of heart infusion media have been described.[5] The addition of carbohydrates or other ingredients results in media used for a variety of purposes. The methodologies for the multiple applications using HIB are outlined in the references.

Principles of the Procedure

Infusion from beef heart and tryptose supply the nutritional requirements for growth of microorganisms in heart infusion media. Sodium chloride maintains the osmotic balance of the medium.

Formula

Bacto™ Heart Infusion Broth

Approximate Formula* Per Liter		
Beef Heart, Infusion from 500 g	10.0	g
Tryptose	10.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 25 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Huntoon. 1918. J. Infect. Dis. 23:169.
2. U.S. Food and Drug Administration. 2001. FDA bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
3. Vanderzant and Splittstoesser (ed.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. Ruoff. 1995. *In* Murray, Baron Pfaller, Tenover and Yolken (ed.)., Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. Atlas. 1997. Handbook of microbiological media, 2nd ed. CRC Press, Inc., Boca Raton, Fla.

Availability

Bacto™ Heart Infusion Broth

BAM CCAM COMPF EP

Cat. No.	238400	Dehydrated – 500 g
	238100	Dehydrated – 2 kg

User Quality Control

Identity Specifications

Bacto™ Heart Infusion Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.5% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 2.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Bacto™ Heart Infusion Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good
Streptococcus pneumoniae	6305	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

Uninoculated Tube

Escherichia coli ATCC™ 25922

Hektoen Enteric Agar

Intended Use

Hektoen Enteric (HE) Agar is a modrately selective medium used in qualitative procedures for the isolation and cultivation of gram-negative enteric microorganisms, especially *Shigella*, from a variety of clinical and nonclinical specimens.

Summary and Explanation

Through the years many media have been devised for the isolation of enteric pathogens. These various formulations have differed in their degree of selectivity for the pathogenic species. Some were designed to isolate and differentiate *Shigella* species whereas others were formulated for the selective isolation of the salmonellae. Media that isolated a broader spectrum of enteric pathogens were less inhibitory to members of the nonpathogenic intestinal flora.

Hektoen Enteric Agar was developed in 1967 by King and Metzger of the Hektoen Institute in order to increase the frequencies of isolation of *Shigella* and *Salmonella* organisms when compared with their recovery on other media frequently utilized in clinical laboratories at that time.[1-3] This medium is considered to be moderately selective, and is particularly useful in the isolation of *Shigella* species. The present formulation differs from that of the original in that sodium desoxycholate has been eliminated and the concentration of bile salts is reduced. Additionally, the peptone concentrations have been increased in order to offset the inhibitory effects of the bile salts.[4]

HE Agar is currently recommended as one of several plating media for the culture of *Enterobacteriaceae* from stool specimens.[5] Foods containing poultry, eggs or dairy products are the most frequent vehicles for foodborne salmonellosis, and a variety of procedures have been developed using Hektoen Enteric Agar as part of the multi-step procedure to isolate *Salmonella*.[6-9]

Principles of the Procedure

The selective nature of Hektoen Enteric Agar is due to the incorporation of bile salts in the formulation. These substances inhibit gram-positive organisms but also can be toxic for some gram-negative strains.

This medium contains three carbohydrates, lactose, sucrose (saccharose) and salicin, for optimal differentiation of enteric pathogens by the color of the colonies and of the medium adjacent to the colonies. The lactose concentration is higher than in many other media used for enterics in order to aid in the visualization of enteric pathogens and minimize the problem of delayed lactose fermentation. Ferric ammonium citrate and sodium thiosulfate in the medium enable the detection of hydrogen sulfide production, thereby aiding in the differentiation process due to the production of black-centered colonies. The indicator system, consisting of acid fuchsin and bromthymol blue, has a lower toxicity than that of many other enteric media, resulting in improved recovery of enteric pathogens.

User Quality Control

Identity Specifications

Difco™ Hektoen Enteric Agar

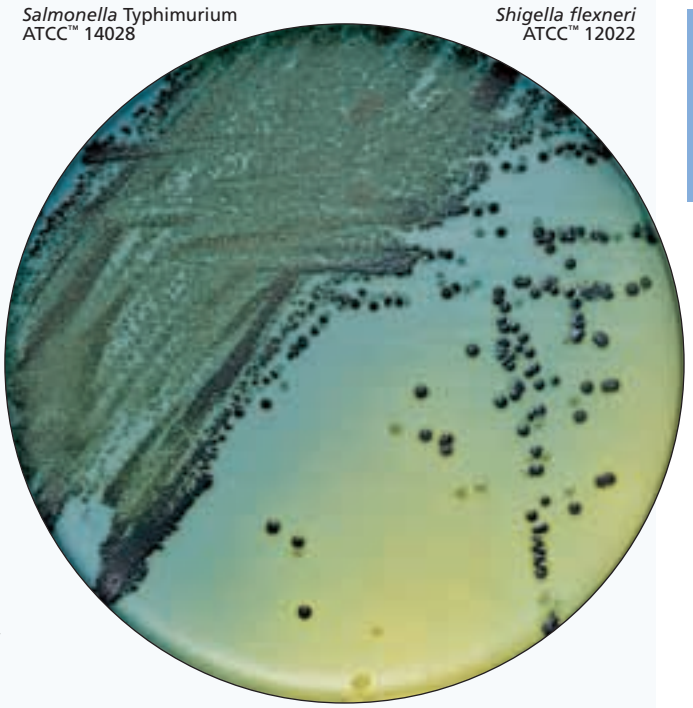
Dehydrated Appearance:	Light beige, may have a slight green cast, free-flowing, homogeneous.
Solution:	7.6% solution, soluble in purified water upon boiling. Solution is brown with greenish cast, slightly opalescent.
Prepared Appearance:	Green with yellowish cast, slightly opalescent.
Reaction of 7.6% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Difco™ Hektoen Enteric Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–
Escherichia coli	25922	10^2-3×10^2	Partial inhibition	Salmon-orange, may have bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-3×10^2	Good	Greenish blue, w/black centers
Shigella flexneri	12022	10^2-3×10^2	Good	Greenish blue



Formula

Difco™ Hektoen Enteric Agar

Approximate Formula* Per Liter		
Proteose Peptone	12.0	g
Yeast Extract	3.0	g
Bile Salts No. 3	9.0	g
Lactose	12.0	g
Saccharose	12.0	g
Salicin	2.0	g
Sodium Chloride	5.0	g
Sodium Thiosulfate	5.0	g
Ferric Ammonium Citrate	1.5	g
Agar	14.0	g
Bromthymol Blue	65.0	mg
Acid Fuchsin	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 76 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat to boiling with frequent agitation to dissolve completely. Do not overheat. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and use immediately.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours.

Expected Results

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Limitation of the Procedure

Proteus species may resemble salmonellae or shigellae. Further testing should be conducted to confirm the presumptive identification of organisms isolated on this medium.

References

1. King and Metzger. 1967. Abstr. M99, p. 77. Bacteriol. Proc. Am. Soc. Microbiol. 1967.
2. King and Metzger. 1968. Appl. Microbiol. *16*:577.
3. King and Metzger. 1968. Appl. Microbiol. *16*:579.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. l. Williams & Wilkins, Baltimore, Md.
5. Murray, Baron, Jorgensen, Landry and Pfaller, (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. Wehr and Frank. (ed.). 2004. Standard Methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
7. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
9. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Hektoen Enteric Agar

AOAC BAM BS12 CCAM CMPH2 COMPF MCM9 SMD

Cat. No.	285340	Dehydrated – 500 g
	285310	Dehydrated – 2 kg
	285320	Dehydrated – 10 kg

BBL™ Hektoen Enteric Agar

AOAC BAM BS12 CCAM CMPH2 COMPF MCM9 SMD

United States and Canada

Cat. No.	221365	Prepared Plates – Pkg. of 20*
	221366	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254009	Prepared Plates – Pkg. of 20*
	254075	Prepared Plates – Ctn. of 120*

Mexico

Cat. No.	224450	Prepared Plates – Pkg. of 10*

BBL™ Hektoen Enteric Agar//Salmonella Shigella Agar

Cat. No. 297426 Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ Hektoen Enteric Agar//XLD Agar

Cat. No. 295646 Prepared **I Plate**™ Dishes – Pkg. of 20*

**Store at 2-8°C.*

Hemo (Haemophilus) Identification (ID) QUAD Plate

Intended Use

The Hemo (Haemophilus) Identification (ID) QUAD Plate (with growth factors) is used for the differentiation and identification of *Haemophilus* species based on requirements for growth factors X and/or V and hemolytic reactions.

Summary and Explanation

In 1917, Davis reported that bacteria causing influenza required two factors for *in vitro* growth, one from hemoglobin and another that the organism derived through a satellite relationship with *Staphylococcus aureus*.[1] In 1921, Thjotta and Avery described the growth factor from blood as X factor and the other as V factor.[2] The X factor was found in hemin and later identified as protoporphyrin IX, or protoheme, and V factor was identified as nicotinamide adenine dinucleotide (NAD).[3,4]

Pathogenic *Haemophilus* species may be differentiated and presumptively identified by determining *in vitro* growth requirements for X and/or V factors and by determining hemolytic reactions.[5]

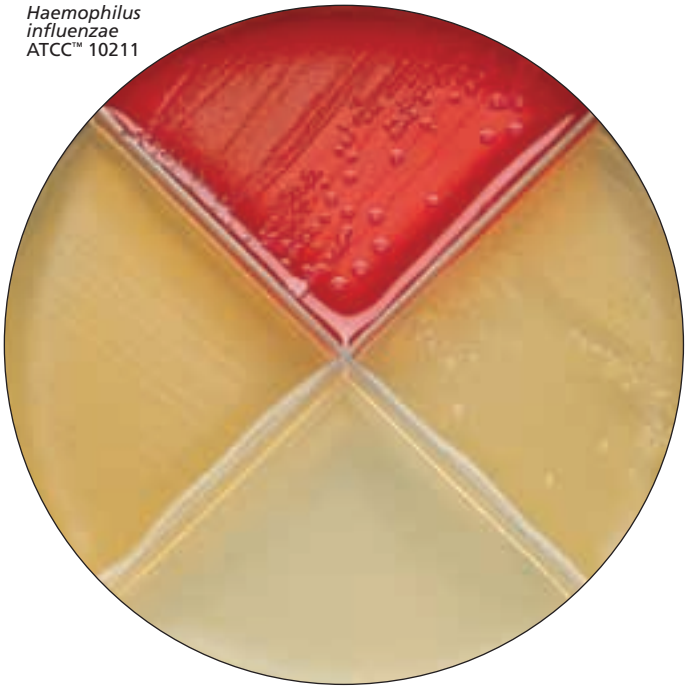
Principles of the Procedure

The Hemo ID QUAD Plate (with growth factors) is a four-sectored plate that contains Brain Heart Infusion Agar in quadrants I, II and III and Blood Agar Base in quadrant IV. Quadrant I is enriched with hemin to supply X factor. Quadrant II is enriched with **BBL**™ **IsoVitaleX**™ Enrichment to supply V factor and other nutrients, such as thiamine and cysteine, which stimulate the growth of *Haemophilus* species. Quadrant III contains both hemin and **IsoVitaleX** Enrichment. Quadrant IV is supplemented with NAD to supply V factor and horse blood to provide X factor and demonstrate hemolytic reactions.

A *Haemophilus* isolate that grows on quadrants III and IV, but fails to grow on quadrants I and II exhibits a requirement for both X and V factors. An isolate that fails to grow on quadrant I, grows on quadrants II, III and IV indicates a requirement only for V factor. Alternatively, an isolate that fails to grow on quadrant II, but grows on quadrants I, III and IV indicates a requirement only for X factor.

Procedure

The initial specimens should be inoculated onto **BBL** Chocolate II Agar or another suitable medium and incubated for 18-24 hours in a CO_2-enriched atmosphere. Choose one or two well-isolated colonies that resemble *Haemophilus* species and perform a Gram stain to confirm that the isolate is a gram-negative rod or coccobacillus. To prepare inoculum, suspend several well-isolated colonies of the test organism from an 18-24 hour plate culture into 5 mL of distilled water or **Trypticase**™ Soy Broth or other suitable medium and adjust the turbidity to a 0.5 McFarland turbidity standard. Dilute 10^{-1}. Inoculate each quadrant with one loopful of diluted specimen and streak to obtain isolated colonies. To prevent carry-over of growth factors, sterilize the loop between inoculations of each quadrant.



Haemophilus influenzae ATCC™ 10211

Alternatively, suspend one or two colonies in 5 mL of distilled water or **Trypticase** Soy Broth or other suitable medium and vortex to mix. Inoculate each quadrant of the plate with one loopful of the diluted specimen and streak to obtain isolated colonies, sterilizing the loop between inoculation of each quadrant.

Do not inoculate plates directly from the chocolate plate. Inoculum must be diluted as described above.

Invert the plates (agar side up) and incubate them in a CO_2-enriched atmosphere at 35°C for 24 hours and examine for growth.

Expected Results

After 24 hours of incubation, the plates should show growth or no growth, depending on X and V factor requirements. The isolate should grow in quadrants III and IV. In quadrant IV, the isolated colonies may have zones of hemolysis, depending on the species isolated.

The following table shows the expected growth results for various *Haemophilus* spp.

Growth on Quadrants*/ Factor(s) Present

	I (X)	II (V)	III (XV)	IV HEMOLYSIS
H. influenzae	–	–	+	–
H. haemolyticus	–	–	+	+
H. parainfluenzae	–	+	+	–
H. parahaemolyticus	–	+	+	+
*H. aphrophilus***	+	+	+	–
H. paraphophilus	–	+	+	–
H. segnis	–	+	+	–
H. ducreyi	+	–	+	–

** + = growth or hemolysis*
– = no growth or no hemolysis

*** X factor requirement occasionally may be observed on primary isolation but will be lost on subculture. Gram staining, biochemical tests and/or additional identification procedures should be performed to confirm findings.*[4-6]

References

1. Davis. 1917. J. Infect. Dis. *21*:392.
2. Thjotta and Avery. 1921. J. Exp. Med. *34*:97.
3. Lwoff and Lwoff. 1937. Proc. Roy. Soc. (London) *122*:352.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
6. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.

Availability

BBL™ Hemo (Haemophilus) Identification (ID) QUAD Plate

MCM9

United States and Canada
Cat. No. 297890 Prepared Plates (QUAD) – Pkg. of 10*

Japan
Cat. No. 251142 Prepared Plates (QUAD) – Pkg. of 10*

**Store at 2-8°C.*

Herrold's Egg Yolk Agar with Mycobactin J and ANV
Herrold's Egg Yolk Agar without Mycobactin J with ANV

Intended Use

Herrold's Egg Yolk Agars are used for the selective isolation and differentiation of *Mycobacterium paratuberculosis*. Mycobacteria other than *M. paratuberculosis* will grow on Herrold's Egg Yolk Agar (w/o Mycobactin), while *M. paratuberculosis* will not grow unless the growth factor, Mycobactin, is provided.

Summary and Explanation

Paratuberculosis (Johne's disease) is a chronic granulomatous enteritis of ruminants first described in 1826.[1] Johne and Frothingham further described the disease in 1895 and demonstrated the presence of acid-fast bacilli in affected intestine.[1] The first successful isolation of this organism occurred in 1910.[2] Early nutritional studies demonstrated the stimulatory effect of an ethanol soluble substance obtained from other mycobacteria (e.g., *M. phlei*) called "mycobactin."[3] The organism fails to grow on the medium lacking the growth factor.

Cultivation of *M. paratuberculosis* from fecal specimens is the most frequently used method to diagnose paratuberculosis. Specific procedures are described elsewhere for the cultivation of *M. paratuberculosis* from bovine fecal specimens.[4-7]

Principles of the Procedure

Enzymatic digest of casein provides amino acids and other nitrogenous substances. Beef extract provides additional nitrogenous nutrients, vitamins and minerals required for microbial growth. Sodium chloride maintains osmotic equilibrium. Sodium pyruvate is a source of energy for bacterial metabolism. Egg yolk and glycerol provide fatty acids and other nutrients required for the metabolism of mycobacteria.

Most mycobacteria produce endogenous siderophores (iron binding compounds) called mycobactins and will grow on Herrold's Egg Yolk Agar whether or not mycobactins have been added. *M. paratuberculosis* is unable to grow on media lacking mycobactins. Mycobactin J permits earlier and more abundant growth of *M. paratuberculosis* than other mycobactins.[8, 9]

Amphotericin B (**A**) enhances the selectivity of the medium by inhibiting contaminating fungi. Nalidixic acid (**N**) inhibits contaminating gram-negative organisms and vancomycin (**V**) inhibits contaminating gram-positive organisms. Malachite green is included to help control contaminants and enhance the visibility of colonies.

Procedure

1. Weigh out 1-2 g of fecal sample.
2. Process specimen using the **MycoPrep**™ Specimen Digestion/Decontamination Kit or a recommended procedure[4-7] to remove or kill contaminating organisms other than mycobacteria.
3. Inoculate tubes of Herrold's Egg Yolk ANV Agar with and without Mycobactin J with no more than 0.25 mL (5-7 drops from a sterile transfer pipette) of the final processed and decontaminated material.
4. Incubate tubes at 35-37°C in a slanted position with the caps loose.
5. Tighten caps when medium surface is dry (1-2 weeks) and place in an upright position in the incubator.
6. Read and evaluate tubes for growth and contamination every week for up to 16 weeks.

Expected Results

Colonies appearing should be evaluated for typical acid-fastness and morphological appearance of *M. paratuberculosis*. *M. paratuberculosis*-like colonies should not appear on the medium lacking Mycobactin J.

To confirm Mycobactin J dependency, suspend suspected colonies in 0.5 mL autoclaved purified water so that the turbidity is equivalent to a No. 1 McFarland nephelometer standard. Dilute the specimen 100-fold in autoclaved purified water. Inoculate 0.1 mL of the suspension onto a single tube of Herrold's Egg Yolk ANV Agar with Mycobactin J and 0.1 mL onto a single tube of the medium without Mycobactin J. Incubate tubes for 1 week with loosened caps at 35-37°C. Tighten caps and re-incubate. Examine tubes weekly for the presence of growth. Acid-fast cultures that grow only in the presence of Mycobactin J are identified as *M. paratuberculosis*.

Limitations of the Procedure

Subcultures of *M. paratuberculosis* may occasionally lose their dependency on Mycobactin. Reduced growth of the organism may be observed after multiple transfers.

References

1. Chiodini, van Kruinigen and Merkal. 1984. Cornell Vet. *74*: 218.
2. Twort. 1910. Proc. R. Soc. Lond. Ser. B. *83*: 156.
3. Twort and Ingram. 1912. Proc. R. Soc. Lond. Ser. B. *84*: 517.
4. Whipple, Callihan and Jarnagin. 1991. J. Vet. Diagn. Invest. *3*: 368.
5. Whitlock, Rosenberger and Spencer. 1989. Proc. Annu. Meet. U.S. Animal Health Assn. *93*: 382.
6. Whitlock and Sweeney. 1990. Proc. Annu. Meet. Livestock Conservation Inst., p. 24.
7. Stabel. 1997. J. Vet. Diagn. Invest. *9*: 375.
8. Merkal and McCullough. 1982. Curr. Microbiol. *7*: 333.
9. Thoen and Baum. 1988. J. Am. Vet. Med. Assoc. *192*: 1609.

Availability

BBL™ Herrold's Egg Yolk Agar with Mycobactin J and ANV

Cat. No.	222232	Prepared Slants – Pkg. of 10*
	222233	Prepared Slants – Ctn. of 100*

BBL™ Herrold's Egg Yolk Agar without Mycobactin J with ANV

Cat. No.	222240	Prepared Slants – Pkg. of 10*
	222241	Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

ISP Medium 1 • ISP Medium 2 • ISP Medium 4

Intended Use

ISP Medium 1, ISP Medium 2 and ISP Medium 4 are used for characterizing *Streptomyces* species according to the International Streptomyces Project (ISP).[1]

Summary and Explanation

ISP media were developed by Difco Laboratories for the International Streptomyces Project (ISP) in order to select stable properties and reproducible procedures for characterization of *Streptomyces* species.[1]

ISP Medium 1 is also referred to as Tryptone-Yeast Extract Broth.

ISP Medium 2 is also referred to as Yeast Extract-Malt Extract Agar.

ISP Medium 4 is also referred to as Inorganic Salts-Starch Agar.

Principles of the Procedure

Peptone and yeast extract provide nitrogen, vitamins, carbon and amino acids in ISP Medium 1.

Yeast extract and malt extract provide nitrogen, amino acids and vitamins in ISP Medium 2. Dextrose is the carbon source. Agar is the solidifying agent.

ISP Medium 4 is composed of many inorganic salts and soluble starch to provide essential nutrients for organism growth. Agar is the solidifying agent.

Formulae

Difco™ ISP Medium 1

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Yeast Extract	3.0	g

Difco™ ISP Medium 2

Approximate Formula* Per Liter		
Yeast Extract	4.0	g
Malt Extract	10.0	g
Dextrose	4.0	g
Agar	20.0	g

Difco™ ISP Medium 4

Approximate Formula* Per Liter		
Soluble Starch	10.0	g
Dipotassium Phosphate	1.0	g
Magnesium Sulfate USP	1.0	g
Sodium Chloride	1.0	g
Ammonium Sulfate	2.0	g
Calcium Carbonate	2.0	g
Ferrous Sulfate	1.0	mg
Manganous Chloride	1.0	mg
Zinc Sulfate	1.0	mg
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ ISP Medium 1 – 8 g;
   Difco™ ISP Medium 2 – 38 g;
   Difco™ ISP Medium 4 – 37 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Mix thoroughly while dispensing ISP Medium 4.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ ISP Medium 1

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in purified water upon boiling. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 0.8% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ ISP Medium 2

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 3.8% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ ISP Medium 4

Dehydrated Appearance:	White to light beige, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is white to off-white, opaque with precipitate.
Prepared Appearance:	White to off-white, opaque, may have a precipitate.
Reaction of 3.7% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ ISP Medium 1, ISP Medium 2 or ISP Medium 4

Prepare the medium per label directions. Inoculate tubes of prepared ISP Medium 1 with the test organisms and incubate at 30 ± 2°C for up to 96 hours. Inoculate prepared ISP Medium 2 and ISP Medium 4 with the test organisms by placing approximately 0.1 mL of inoculum near the edge of the plate. Five parallel streaks across the plate are made from this 0.1 mL of inoculum, followed by four perpendicular streaks. Incubate inoculated plates at 30 ± 2°C for 48-96 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Streptomyces albus	3004	10^2-10^3	Good
Streptomyces lavendulae	8664	10^2-10^3	Good

Procedure
For details on the use of these media for characterization of *Streptomyces* species, consult the reference.[1] For a complete discussion on the isolation and maintenance of *Streptomyces* species refer to appropriate references.[2,3]

Expected Results
Refer to appropriate references and procedures for results.

References
1. Shirling and Gottlieb. 1966. Int. J. Syst. Bacteriol. *16*:313.
2. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability
Difco™ ISP Medium 1
Cat. No. 276910 Dehydrated – 500 g

Difco™ ISP Medium 2
Cat. No. 277010 Dehydrated – 500 g

Difco™ ISP Medium 4
Cat. No. 277210 Dehydrated – 500 g

Indole Nitrite Medium (Trypticase™ Nitrate Broth)

Intended Use
Indole Nitrite Medium is used for the identification of microorganisms by means of the nitrate reduction and indole tests.

Summary and Explanation
Indole Nitrite Medium was developed to serve the dual role of detecting indole production and nitrate reduction of a wide range of microorganisms. Due to its nutritive content, the medium will support the growth of aerobes, microaerophiles and facultative and obligate anaerobes.

Indole Nitrite Medium can be used for nitrite tests with members of the *Enterobacteriaceae* but is not recommended for the indole test with these organisms since they reduce nitrate to nitrite, which prevents the detection of indole.[1] Tryptophan (**Trypticase**™) 1% Solution is the medium of choice for indole test with enteric bacilli.

User Quality Control

Identity Specifications
BBL™ Indole Nitrite Medium (Trypticase™ Nitrate Broth)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.5% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, trace hazy to hazy.
Prepared Appearance:	Light to medium, yellow to tan, trace hazy to hazy.
Reaction of 2.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response
BBL™ Indole Nitrite Medium (Trypticase™ Nitrate Broth)
Prepare the medium per label directions. Inoculate with fresh broth cultures diluted 1:10 and incubate at 35 ± 2°C for 2 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	RECOVERY	NITRATE	INDOLE
Clostridium perfringens	13124	Good	+	–
Clostridium bifermentans	17836	Good	–	+
Escherichia coli	25922	Good	+	+

Principles of the Procedure
The casein peptone contains tryptophan, which is attacked by certain microorganisms, resulting in the production of indole, detectable by the addition of chemical reagents to 18- to 48-hour cultures. Potassium nitrate serves as the substrate for determining the ability of microorganisms to reduce nitrates to nitrites.

Formula
BBL™ Indole Nitrite Medium (Trypticase™ Nitrate Broth)

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	20.0	g
Disodium Phosphate	2.0	g
Dextrose	1.0	g
Agar	1.0	g
Potassium Nitrate	1.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 25 g of the powder in 1 L of purified water. Add 2 g of agar for use as a motility medium. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in regular test tubes, filing them half full. Autoclave at 121°C for 15 minutes.
4. If the medium is more than 2 days old at the time of use, boil and cool prior to use.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Inoculate the tubes with pure cultures of the organisms being evaluated. Replicate tubes should be inoculated if it is desired to test for the presence of indole or nitrites after incubation

for various lengths of time. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere. The caps of tubes inoculated with obligate anaerobes should be tightened during incubation.

1. Indole Test
   The test for indole may be performed as soon as heavy growth has taken place, usually after 18 to 48 hours of incubation. The test may be performed by any suitable method, such as with Kovacs' reagent (add 0.5 mL, Cat. No. 261185) or Ehrlich's reagent employing *p*-dimethylaminobenzaldehyde.[2] Testing for indole may be made after 24 hours of incubation; if negative, the test should be repeated on another culture incubated for 48 hours.
2. Nitrite Test
   The test for nitrites may be performed at several intervals during the incubation process if replicate tubes were inoculated. The presence of nitrites may be detected by any of several methods.[2] Addition of approximately 5 drops each of sulfanilic acid (Cat. No. 261197) and N, N-dimethyl-1-naphthylamine (Cat. No. 261198) reagents permits the detection of nitrites. If prior tests are negative, a final test should be conducted at 48 hours of incubation.

Expected Results

1. Indole Test
   The production of a pink to red color following addition of the reagent is a positive test for indole formation.
2. Nitrite Test
   A pink to red color develops, after addition of the reagents, if nitrite is present, and indicates that nitrate reduction has occurred. Since some organisms further reduce nitrite to ammonia, add a small amount of zinc dust (Cat. No. 261207) to tubes exhibiting no color. A pink color in this part of the test indicates no nitrate reduction. A colorless reaction indicates that nitrates have been completely reduced.

Consult appropriate references for an explanation of the reactions involved and expected results with specific microorganisms.[3,4]

Limitations of the Procedure

Indole Nitrite Medium should not be used for detecting indole production by members of the *Enterobacteriaceae*. The tubed medium should be boiled for 2 minutes and cooled, without agitation, before use.

References

1. Smith, Rogers and Bettge. 1972. Appl. Microbiol. *43*:423.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Indole Nitrite Medium (Trypticase™ Nitrate Broth)

BAM

Cat. No.	211299	Dehydrated – 500 g
	221655	Prepared Tubes – Pkg. of 10*

Difco™/BBL™ Indole Reagent

Cat. No.	261185	Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate A Reagent

Cat. No.	261197	Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate B Reagent

Cat. No.	261198	Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate C Reagent

Cat. No.	261207	Droppers, 1 g – Ctn. of 50

**Store at 2-8°C.*

Inhibitory Mold Agar
Inhibitory Mold Agar with Gentamicin

Intended Use

Inhibitory Mold Agar, which contains chloramphenicol, is a moderately selective medium used for the isolation of pathogenic fungi. **BBL**™ prepared plates of Inhibitory Mold Agar and Inhibitory Mold Agar with Gentamicin are deep filled to reduce the effects of drying during prolonged incubation.

Summary and Explanation

Inhibitory Mold Agar was formulated by Ulrich as a general medium for the selective isolation and cultivation of the majority of pathogenic fungi.[1]

Principles of the Procedure

The nutritive properties of Inhibitory Mold Agar are provided by the two peptones, derived from casein and animal tissue, and yeast extract, which is a rich source of vitamins. Dextrose, starch and dextrin are energy sources for the metabolism of fungi. Sodium chloride and the metallic salts provide essential ions and minerals. Chloramphenicol is a broad-spectrum antibiotic that inhibits a wide range of gram-positive and gram-negative bacteria. Gentamicin, if present, inhibits gram-negative microorganisms.

User Quality Control

Identity Specifications

BBL™ Inhibitory Mold Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, slightly hazy to hazy.
Prepared Appearance:	Light to medium, yellow to tan, slightly hazy to hazy.
Reaction of 3.6% Solution at 25°C:	pH 6.7 ± 0.2

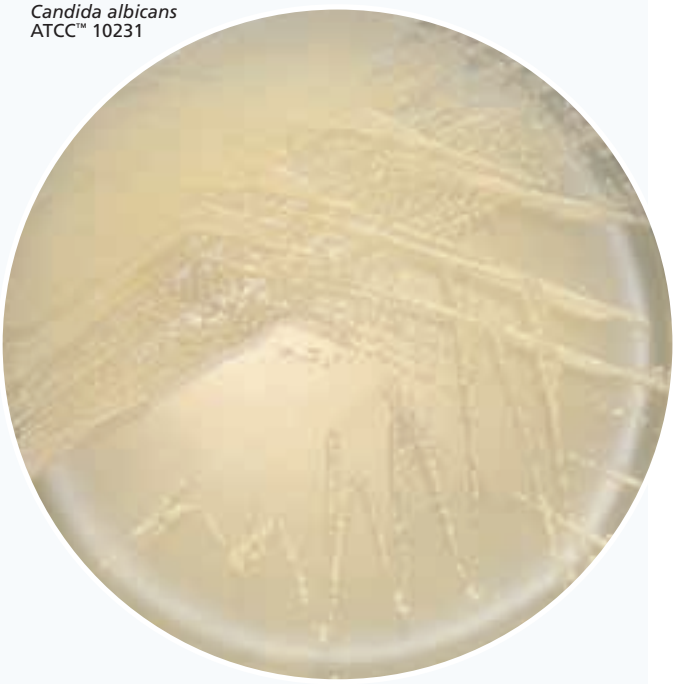
Cultural Response

BBL™ Inhibitory Mold Agar

Prepare the medium per label directions. Inoculate with fresh cultures (undiluted or diluted as described below) and incubate at 25 ± 2°C for 7 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Candida albicans	10231	Undiluted	Good
Escherichia coli	25922	10^4-10^5	Partial to complete inhibition
Trichophyton mentagrophytes	9533	Undiluted	Good

Candida albicans
ATCC™ 10231



Formula

BBL™ Inhibitory Mold Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	3.0	g
Peptic Digest of Animal Tissue	2.0	g
Yeast Extract	5.0	g
Dextrose	5.0	g
Starch	2.0	g
Dextrin	1.0	g
Chloramphenicol	125.0	mg
Sodium Phosphate	2.0	g
Magnesium Sulfate	0.8	g
Ferrous Sulfate	0.04	g
Sodium Chloride	0.04	g
Manganese Sulfate	0.16	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for information about the processing and inoculation of specimens.[2]

For isolation of fungi from potentially contaminated specimens, a nonselective medium should be inoculated along with the selective medium. Incubate the plates at 25-30°C in an inverted position (agar side up) with increased humidity. The tubed slants also should be incubated at 25-30°C. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C.

All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Expected Results

Examine plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Some fungi may be inhibited by the antibiotics in Inhibitory Mold Agar and Inhibitory Mold Agar with Gentamicin.[3]

References

1. Ulrich. 1956. Abstr. M75, p. 87. Bacteriol. Proc. Soc. Am. Bacteriologists. 1956.
2. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Ajello, Georg, Kaplan and Kaufmann. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Inhibitory Mold Agar

BS12 CMPH2 MCM9

Cat. No.	292846	Dehydrated – 500 g
	212254	Dehydrated – 5 lb (2.3 kg)
	297799	Prepared Plates (Deep Fill) – Pkg. of 10*
	298191	Prepared Plates (Deep Fill) – Ctn. of 100*
	297276	Prepared Slants – Pkg. of 10*
	297826	Transgrow-style Bottle – Pkg. of 10*
	297757	Transgrow-style Bottle – Ctn. of 100*

BBL™ Inhibitory Mold Agar with Gentamicin

BS12 CMPH2 MCM9

Cat. No.	297800	Prepared Plates (Deep Fill) – Pkg. of 10*

**Store at 2-8°C.*

Inositol Assay Medium

Intended Use

Inositol Assay Medium is used for determining inositol concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Inositol Assay Medium, a modification of the formula described by Atkin et al.,[1] is used in the microbiological assay of inositol using *Saccharomyces cerevisiae* ATCC™ 9080 (*Saccharomyces uvarum*) as the test organism.

Principles of the Procedure

Inositol Assay Medium is an inositol-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *S. cerevisiae* ATCC 9080. The addition of inositol in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

User Quality Control

Identity Specifications

Difco™ Inositol Assay Medium

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous (may contain small dark particles).
Solution:	6.1% (single strength) solution, soluble in purified water upon boiling. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Light amber, clear, may have a slight precipitate.
Reaction of 6.1% Solution at 25°C:	pH 5.2 ± 0.2

Cultural Response

Difco™ Inositol Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Saccharomyces cerevisiae* ATCC™ 9080 when prepared in single strength and supplemented with inositol. The medium should produce a standard curve using an inositol reference standard at 0.0 to 10.0 µg per 10 mL. Incubate tubes with caps loosened at 25-30°C for 20-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Formula

Difco™ Inositol Assay Medium

Approximate Formula* Per Liter		
Dextrose	100.0	g
Potassium Citrate	10.0	g
Citric Acid	2.0	g
Monopotassium Phosphate	1.1	g
Potassium Chloride	0.85	g
Magnesium Sulfate	0.25	g
Calcium Chloride	0.25	g
Manganese Sulfate	50.0	mg
Ferric Chloride	50.0	mg
DL-Tryptophan	80.0	mg
L-Cystine	0.1	g
L-Isoleucine	0.5	g
L-Leucine	0.5	g
L-Lysine	0.5	g
L-Methionine	0.2	g
DL-Phenylalanine	0.2	g
L-Tyrosine	0.2	g
L-Asparagine	0.8	g
DL-Aspartic Acid	0.2	g
DL-Serine	0.1	g
Glycine	0.2	g
DL-Threonine	0.4	g
L-Valine	0.5	g
L-Histidine	124.0	mg
L-Proline	0.2	g
DL-Alanine	0.4	g
L-Glutamic Acid	0.6	g
L-Arginine	0.48	g
Thiamine Hydrochloride	500.0	µg
Biotin	16.0	µg
Calcium Pantothenate	5.0	mg
Pyridoxine Hydrochloride	1.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 12.2 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense 5 mL amounts into tubes.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL.
6. Autoclave at 121°C for 5 minutes.

Procedure

Remove a loopful of culture from a stock culture slant of *S. cerevisiae* ATCC 9080 and suspend it in 10 mL sterile 0.85% saline. Centrifuge cells at moderate speed for 10 minutes.

Decant the supernatant and resuspend cells in 10 mL 0.85% sterile saline. Wash the cells three times with 10 mL sterile 0.85% saline. After the third wash, resuspend the cells in 10 mL 0.85% saline. Dilute 1 mL of the cell suspension in 1000 mL of sterile 0.85% saline. This diluted suspension is the inoculum. Use 1 drop of inoculum suspension to inoculate each assay flask.

The concentrations of inositol required for the preparation of the standard curve may be prepared by dissolving 200 mg inositol in 100 mL purified water. Mix thoroughly. Dilute 1 mL of this solution with 999 mL purified water to make a final solution containing 2 µg inositol per mL. Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL per flask. Prepare this stock solution fresh daily.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can impact the standard curve readings and cannot always be duplicated. The standard curve is obtained by using inositol at levels of 0.0, 1, 2, 4, 6, 8 and 10 µg per assay flask (10 mL).

Following inoculation, flasks are incubated at 25-30°C for 20-24 hours. Place flasks in the refrigerator for 15-30 minutes to stop growth. Growth is measured turbidimetrically using any suitable spectrophotometer.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ± 10% from the average. Use the results only if two-thirds of the values do not vary more than ± 10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be grown and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

Reference

1. Atkin, Schultz, Williams and Frey. 1943. End. & Eng. Chem., Ann. Ed. *15*:141.

Availability

Difco™ Inositol Assay Medium

Cat. No. 212222 Dehydrated – 100 g*

**Store at 2-8°C.*

Jordan's Tartrate Agar

Intended Use

Jordan's Tartrate Agar is used as an aid in the identification of members of the *Enterobacteriaceae* on the basis of tartrate utilization.

Summary and Explanation

Jordan's Tartrate Agar was formulated by Jordan and Harmon for the detection of sodium tartrate fermentation, a test used to differentiate *Salmonella typhi* (now *S. enterica* subsp. *enterica* serotype Typhi) and *S. schottmuelleri* (now *S. enterica* subsp. *enterica* serotype Paratyphi B).[1] Edwards and Ewing later reported tartrate utilization useful for the differentiation of monophasic and diphasic Arizona (now *S. enterica* subsp. *arizonae*) cultures.[2]

Jordan's Tartrate Agar is particularly helpful in the differentiation of *S. enterica* subsp. *enterica* serotype Paratyphi A, which does not utilize tartrate, from other strains of salmonellae most of which are capable of utilizing tartrate.[3,4]

Principles of the Procedure

Utilization of the organic salt, sodium tartrate, may be used to differentiate enteric bacilli. Tartrate fermentation acidifies the medium and is indicated by the development of a yellow color in the lower portion of the tube. Phenol red is incorporated as an indicator of acid production.

Procedure

Prior to inoculation of Jordan's Tartrate Agar, the organism to be tested must have been previously isolated on some other suitable solid medium. The use of a pure culture is essential to correct performance of the test.

Using a sterile inoculating needle, pick the center of a well-isolated colony from a young culture and inoculate the medium by stabbing deeply into the column of medium. Replace the cap loosely. Incubate the tubes at 35 ± 2°C for 24-48 hours in an aerobic atmosphere.

Expected Results

The test is positive if the lower portion of the medium has turned yellow. If there is no change in the color of the medium, the test is negative.

Limitation of the Procedure

Tartrate utilization is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, biochemical and serological tests. Appropriate texts should be consulted for additional information.[3-5]

References

1. Jordan and Harmon. 1928. J. Infect. Dis. 42:238.
2. Edwards and Ewing. 1955. Identification of *Enterobacteriaceae*. Burgess Publishing Company, Minneapolis, Minn.
3. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Jordan's Tartrate Agar

USDA

Cat. No. 221889 Prepared Agar Deeps (K size tubes) – Pkg. of 10*

**Store at 2-8°C.*

KF Streptococcus Agar TTC Solution 1%

Intended Use

KF Streptococcus Agar is used with TTC Solution 1% in isolating and enumerating fecal streptococci.

Summary and Explanation

Kenner et al. developed KF (Kenner Fecal) Streptococcal Agar with TTC for use in detecting streptococci in surface waters by direct plating or by the membrane filtration method.[1] These investigators compared the performance of their formulation to other media used for enumerating fecal streptococci and achieved greater recoveries with KF Streptococcal Agar. The medium is recommended for use in determining counts of fecal streptococci in water.

TTC (2, 3, 5-Triphenyl Tetrazolium Chloride) in aqueous 1% solution may be added to various agar and fluid media to lend color to cells and to colonies on agar or on membranes, thereby facilitating the detection of growth.

Principles of the Procedure

Peptone provides a source of nitrogen, amino acids and carbon. Yeast extract is a source of trace elements, vitamins and amino acids. Maltose and lactose are fermentable carbohydrates and carbon sources. Sodium azide is a selective agent. Bromcresol purple is an indicator dye.

User Quality Control

Identity Specifications

Difco™ KF Streptococcus Agar

Dehydrated Appearance:	Light greenish-beige, free-flowing, homogeneous.
Solution:	7.64% solution, soluble in purified water upon boiling. Solution is light purple, very slightly to slightly opalescent.
Prepared Appearance:	Light purple, very slightly to slightly opalescent.
Reaction of 7.64% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ TTC (powder)

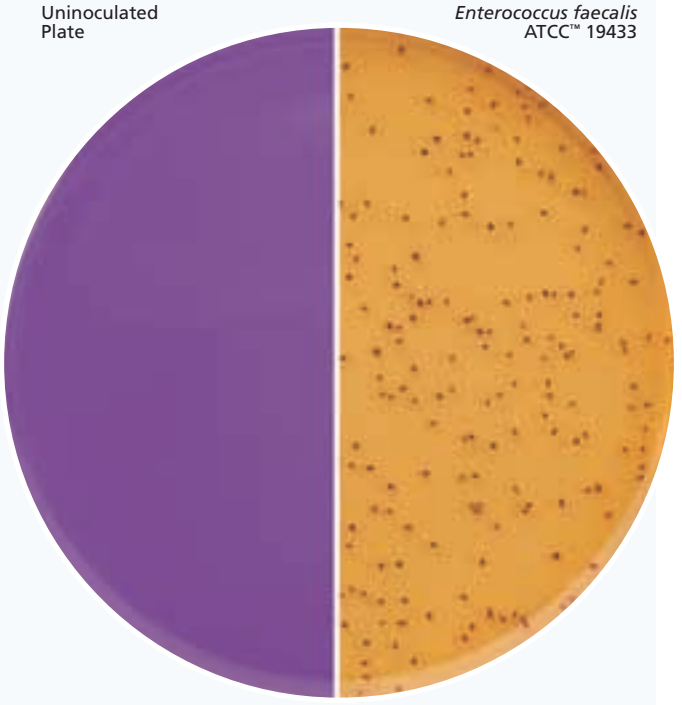
Appearance:	White to slightly yellow, free-flowing, homogeneous.
Solution:	1% solution, soluble in purified water. Solution is colorless to very slight amber, clear.

Cultural Response

Difco™ KF Streptococcus Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 46-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterobacter aerogenes	13048	3×10^2-10^3	Marked to complete inhibition	–
Enterococcus faecalis	19433	30-300	Good	Red to pink centers
Enterococcus faecalis	29212	30-300	Good	Red to pink centers
Escherichia coli	25922	3×10^2-10^3	Marked to complete inhibition	–



H–K

TTC is used as a redox indicator in culture media. It is colorless in the oxidized form and is reduced to formazan, an insoluble red pigment, by actively growing microbial cells. The reduction of TCC is irreversible; it is not reoxidized by air once it is reduced to the red formazan.[2]

In this medium, the addition of 1% triphenyltetrazolium chloride (TTC) causes enterococci to develop a deep red color.

Formula

Difco™ KF Streptococcus Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	10.0	g
Yeast Extract	10.0	g
Sodium Chloride	5.0	g
Sodium Glycerophosphate	10.0	g
Maltose	20.0	g
Lactose	1.0	g
Sodium Azide	0.4	g
Bromcresol Purple	15.0	mg
Agar	20.0	g

TTC Solution 1%

Formula Per 100 mL		
2, 3, 5-Triphenyltetratolium Chloride	1.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 76.4 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
2. Heat an additional 5 minutes. Avoid overheating which could decrease the productivity of the medium. DO NOT AUTOCLAVE.
3. Aseptically add 10 mL TTC Solution 1% to the medium cooled to 50°C. Mix well.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Pour Plate Technique

1. Prepare appropriate dilutions of the test material.
2. Place the selected volume of sample in a Petri dish.
3. Pour 15 mL of the prepared medium at 45-50°C into each plate.
4. Thoroughly mix the medium and sample to uniformly disperse the organisms.
5. Allow the agar to solidify.
6. Incubate plates in the inverted position at 35 ± 2°C for 46-48 hours.

Membrane Filter Procedure

1. Filter a suitable volume of sample through a sterile membrane.
2. Place the inoculated membrane filter on the solidified agar in the Petri dish, inoculum side up.
3. Incubate the plates, inverted, at 35 ± 2°C for 46-48 hours.

Expected Results

Enterococci will appear as red or pink colonies. The use of a stereoscopic microscope with 15× magnification can aid in counting colonies.

Limitations of the Procedure

1. Many strains of *S. bovis* and *S. equinus* are inhibited by azide.
2. Overheating may lower the pH, causing a decrease in the productivity of the medium.

References

1. Kenner, Clark and Kabler. 1961. Appl. Microbiol. 9:15.
2. Kelly and Fulton. 1953. Am. J. Clin. Pathol. 23:512.

Availability

Difco™ KF Streptococcus Agar

Cat. No. 249610 Dehydrated – 500 g

Difco™ TTC Solution 1%

Cat. No. 231121 Tube – 30 mL
264310 Bottle – 25 g

**Store at 2-8°C.*

KF Streptococcus Broth

Intended Use

KF Streptococcus Broth is used for isolating fecal streptococci.

Summary and Explanation

Kenner et al. developed KF (Kenner Fecal) Streptococcal Broth for the detection and enumeration of enterococci in waters.[1,2] They found that this formulation was superior to other liquid media in the recovery of enterococci in Most Probable Number (MPN) test systems. The medium is not specific for presumptive identification of group D streptococci. Other tests are required.[2-4]

Principles of the Procedure

Peptone provides a source of nitrogen, amino acids and carbon. Yeast extract is a source of trace elements, vitamins and amino acids. Maltose and lactose are the fermentable carbohydrates and carbon sources. Sodium azide is the selective agent. Bromcresol purple is the indicator dye.

The addition of 1% TTC (2,3,5-Triphenyl Tetrazolium Chloride), in the membrane filter procedure, causes the enterococci to have a deep red color as a result of tetrazolium reduction to an acid azo dye.

User Quality Control

Identity Specifications

Difco™ KF Streptococcus Broth

Dehydrated Appearance:	Light greenish-beige, free-flowing, homogeneous.
Solution:	5.64% solution, soluble in purified water upon boiling. Solution is reddish to light purple, clear to very slightly opalescent.
Prepared Appearance:	Purple, clear to very slightly opalescent.
Reaction of 5.64% Solution at 25°C:	pH 7.2 ± 0.2

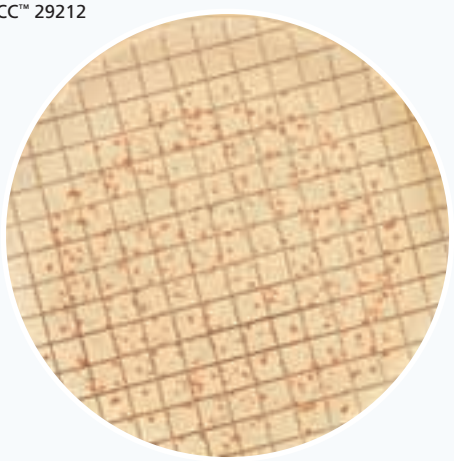
Cultural Response

Difco™ KF Streptococcus Broth

Prepare the medium per label directions. Supplement with TTC Solution 1%. Using the membrane filter technique, inoculate and incubate at 35 ± 1°C in an atmosphere saturated with water vapor for 46-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterobacter aerogenes	13048	3×10^{2}-10^{3}	Inhibition	–
Enterococcus faecalis	19433	30-200	Good	Red
Enterococcus faecalis	29212	30-200	Good	Red
Escherichia coli	25922	3×10^{2}-10^{3}	Inhibition	–

Enterococcus faecalis
ATCC™ 29212



Formula

Difco™ KF Streptococcus Broth

Approximate Formula* Per Liter

Proteose Peptone No. 3	10.0	g
Yeast Extract	10.0	g
Sodium Chloride	5.0	g
Sodium Glycerophosphate	10.0	g
Maltose	20.0	g
Lactose	1.0	g
Sodium Azide	0.4	g
Bromcresol Purple	15.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

MPN Procedure

1. For an inoculum of 1 mL or less, suspend 56.4 g of the powder in 1 L of purified water. Mix thoroughly.
   For an inoculum of 10 mL, suspend 84.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. For an inoculum of 1 mL or less, dispense 10 mL amounts into culture tubes.
   For an inoculum of 10 mL, dispense 20 mL amounts into culture tubes.
4. Autoclave at 121°C for 10 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Membrane Filter Procedure

1. Suspend 56.4 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in 100 mL amounts into flasks and autoclave at 121°C for 10 minutes.
4. Cool to 60°C and add 1 mL TTC Solution 1% per 100 mL of medium.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

MPN Procedure

1. Inoculate tubes of the KF Streptococcus Broth with the appropriate amount of inoculum.
2. Incubate tubes at 35 ± 1°C, with loosened caps, for 46-48 hours.

Membrane Filter Procedure

1. Place a sterile absorbent pad in each sterile Petri dish.
2. Saturate the pads with the sterile medium containing TTC.
3. Place an inoculated membrane filter, inoculated side up, on the saturated pad.
4. Incubate at 35 ± 1°C in an atmosphere saturated with water vapor for 46-48 hours.

Expected Results

MPN Procedure

MPN tubes positive for enterococci are turbid with growth that appears yellow in color and does not produce foaming. When foaming occurs, confirmation for enterococci should be made by Gram staining.

Membrane Filter Procedure

All red or pink colonies visible with 15× magnification are counted as enterococci colonies.

Limitations of the Procedure

1. Many strains of *S. bovis* and *S. equinus* are inhibited by azide.
2. Overheating may lower the pH, resulting in a decrease in productivity of the medium.

References

1. Kenner, Clark and Kabler. 1960. Am. J. Public Health *50*:1553.
2. Kenner, Clark and Kabler. 1961. Appl. Microbiol. *9*:15.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Facklam and Moody. 1970. Appl. Microbiol. *20*:245.

Availability

Difco™ KF Streptococcus Broth

Cat. No. 212226 Dehydrated – 500 g

Difco™ TTC Solution 1%

Cat. No. 231121 Tube – 30 mL
264310 Bottle – 25 g

Kligler Iron Agar

Intended Use

Kligler Iron Agar is used for the differentiation of members of the *Enterobacteriaceae* on the basis of their ability to ferment dextrose and lactose and to liberate sulfides.

Summary and Explanation

In 1911, Russell described a new double sugar tube medium for the isolation of typhoid bacilli from urine and feces.[1] Six years later, Kligler developed a simple lead acetate medium for the differentiation of the typhoid-paratyphoid group.[2] Subsequently, Kligler evaluated culture media used in the isolation and differentiation of typhoid, dysentery and allied bacilli and endorsed Russell's medium.[3] Bailey and Lacey substituted phenol red for the Andrade indicator previously used as a pH indicator.[4]

The current formulation of Kligler Iron Agar combines features of Kligler's lead acetate medium with those of Russell's double sugar agar.

Principles of the Procedure

Kligler Iron Agar, in addition to casein and meat peptones, contains lactose and dextrose which enable the differentiation of species of enteric bacilli due to color changes of the phenol

User Quality Control

Identity Specifications

BBL™ Kligler Iron Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is medium to dark, orange to red, with or without a tint of brown, clear to slightly hazy.
Prepared Appearance:	Medium to dark, orange to red, with or without a tint of brown, clear to slightly hazy.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Kligler Iron Agar

Prepare the medium per label directions. Stab inoculate with fresh cultures and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	RECOVERY	SLANT	BUTT	H_2S
Escherichia coli	25922	Good	Acid	Acid with gas	–
Morganella morganii	8019	Good	Alkaline	Acid with or without gas	–
Pseudomonas aeruginosa	27853	Good	Alkaline	Alkaline without gas	–
Salmonella enterica subsp. *enterica* serotype Typhi	19430	Good	Alkaline	Acid without gas	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	Alkaline	Acid with gas	+
Shigella flexneri	12022	Good	Alkaline	Acid without gas	–

Uninoculated Tube | *Echerichia coli* ATCC™ 25922 | *Morganella morganii* ATCC™ 8019 | *Salmonella* Typhimurium ATCC™ 14028

red pH indicator in response to the acid produced during the fermentation of these sugars. The dextrose concentration is only 10% of the lactose concentration. The combination of ferric ammonium citrate and sodium thiosulfate enables the detection of hydrogen sulfide production.

Lactose nonfermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid produced by the fermentation of the small amount of dextrose. When the dextrose supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids. The reversion does not occur in the anaerobic environment in the butt, which remains acid (yellow butt). Lactose fermenters produce yellow slants and butts because enough acid is produced in the slant to maintain an acid pH under aerobic conditions. Organisms incapable of fermenting either carbohydrate produce red slants and butts.

Hydrogen sulfide production is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar.

Formula

BBL™ Kligler Iron Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Lactose	10.0	g
Dextrose	1.0	g
Sodium Chloride	5.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Thiosulfate	0.5	g
Agar	15.0	g
Phenol Red	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Cool in a slanted position such that deep butts are formed. For best results, the medium should be used on the date of preparation or melted and resolidified before use.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To inoculate, carefully touch the center of an isolated colony on an enteric plated medium with a cool, sterile needle, stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant. Several colonies from each primary plate should be studied separately, since mixed infections may occur. Incubate tubes with loosened caps for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

To enhance the alkaline condition in the slant, free exchange of air must be permitted through the use of a loose closure. If the tube is tightly closed, an acid reaction (caused solely by dextrose fermentation) will also involve the slant.

Expected Results

After incubation, record the reaction in the slant and butt, noting gas formation and hydrogen sulfide production.

Typical reactions produced by members of the *Enterobacteriaceae* (majority of the species in the particular genus) are presented in the following table.[5]

	SLANT	BUTT	GAS	H_2S
Citrobacter	Alkaline	Acid	+	+ or –
Edwardsiella	Alkaline	Acid	+	+
Escherichia coli	Acid	Acid	+	–
Enterobacter	Acid*	Acid	+	–
Morganella	Alkaline	Acid	±	–
Proteus	Alkaline or Acid	Acid	+	+
Providencia	Alkaline	Acid	±	–
Salmonella	Alkaline	Acid	+	+
Shigella	Alkaline	Acid	–	–

**May revert to alkaline even though lactose fermented (*E. aerogenes*).*

References

1. Russell. 1911. J. Med. Res. 25:217.
2. Kligler. 1917. Am. J. Public Health. 7:1041.
3. Kligler. 1918. J. Exp. Med. 28:319.
4. Bailey and Lacy. 1927. J. Bacteriol. 13:183.
5. Ewing. 1986. Edwards and Ewing's identification of the *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc. New York, N.Y.

Availability

BBL™ Kligler Iron Agar

BAM CCAM CMPH2 COMPF ISO MCM9

Cat. No.	211317	Dehydrated – 500 g
	220896	Prepared Slants – Pkg. of 10*
	220897	Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

Koser Citrate Medium

Intended Use

Koser Citrate Medium is used for differentiating *Escherichia coli* from *Enterobacter aerogenes* based on citrate utilization.

Summary and Explanation

In 1923, the work of Koser demonstrated that coli-aerogenes bacteria could be differentiated by their use of certain salts of organic acids.[1] Koser found that the sodium salt of citric acid (sodium citrate) is used as a source of carbon by *E. aerogenes* and not by *E. coli*. Biochemical identification schemes for identifying *E. coli* frequently include Koser citrate.

E. coli is an important member of the coliform group of bacteria. The coliforms are described as aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products.[2-5] Presumptive identification is confirmed by performing biochemical tests that specifically identify *E. coli*.

Principles of the Procedure

Koser Citrate Medium is prepared with chemically pure salts and tested to determine that no sources of carbon (other than sodium citrate) or nitrogen (other than ammonium salts) are present. Bacteria that are able to use citrate as their carbon source will grow in the medium and cause turbidity.

Formula

Difco™ Koser Citrate Medium

Approximate Formula* Per Liter		
Sodium Ammonium Phosphate	1.5	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.2	g
Sodium Citrate	3.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 5.7 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Transfer growth from a single colony or a loopful of liquid suspension and inoculate the broth medium.
2. Incubate at 35 ± 2°C for 18-24 hours.

User Quality Control

Identity Specifications

Difco™ Koser Citrate Medium

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	0.57% solution, soluble in purified water. Solution is colorless, clear.
Prepared Appearance:	Colorless, clear.
Reaction of 0.57% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Koser Citrate Medium

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterobacter aerogenes	13048	10^3	Good
Escherichia coli	25922	10^3	Marked to complete inhibition

Expected Results

Positive: .....Turbidity
Negative: .....Clear, no turbidity

References

1. Koser. 1923. J. Bacteriol. 8:493.
2. Wehr and Frank (ed.). 2004. Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Horwitz and Garcia (ed.). 2004 (update, 2007). Official methods of analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, Md.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Koser Citrate Medium

AOAC BAM COMPF SMD

Cat. No. 215100 Dehydrated – 500 g

LB Agar, Lennox • LB Broth, Lennox

Intended Use

LB Agar, Lennox and LB Broth, Lennox are used for maintaining and cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

LB Agar, Lennox and LB Broth, Lennox are nutritionally rich media developed by Lennox for the growth and maintenance of pure cultures of recombinant strains of *E. coli*.[1] These strains are generally derived from *E. coli* K12, which are deficient in B vitamin production. This strain of *E. coli* has been further modified through specific mutation to create an auxotrophic strain that is not capable of growth on nutritionally deficient media.

LB Agar, Lennox provides all the nutritional requirements of these organisms. LB Agar, Lennox contains half the sodium chloride level of the Miller formulation of LB Agar.[2] This allows the researcher to select the optimal salt concentration for a specific strain.

LB Broth, Lennox contains ten times the sodium chloride level of Luria Broth Base, Miller and one half of that found in LB Broth, Miller.[3] This allows the researcher to select the optimal salt concentration for a specific strain. If desired, the medium may be aseptically supplemented with glucose to prepare the complete medium described by Lennox.

User Quality Control

Identity Specifications

Difco™ LB Agar, Lennox

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent.
Prepared Appearance:	Medium amber, very slightly to slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ LB Broth, Lennox

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Very light amber, clear to very slightly opalescent.
Reaction of 2.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ LB Agar, Lennox or LB Broth, Lennox

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AGAR	RECOVERY BROTH
Escherichia coli	33694 (HB101)	10^2-3×10^2	Good	Good
Escherichia coli	39403 (JM103)	10^2-3×10^2	Good	Good
Escherichia coli	53868 (DH5)	10^2-3×10^2	Good	Good

Principles of the Procedure

Peptone provides nitrogen and carbon. Vitamins (including B vitamins) and certain trace elements are provided by yeast extract. Sodium ions for transport and osmotic balance are provided by sodium chloride. Agar is the solidifying agent in LB Agar, Lennox.

Formulae

Difco™ LB Agar, Lennox

Approximate Formula* Per Liter

Tryptone	10.0	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

Difco™ LB Broth, Lennox

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ LB Agar, Lennox – 35 g;
   Difco™ LB Broth, Lennox – 20 g.
   Mix thoroughly.
2. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[1-4]

Expected Results

After sufficient incubation, the agar medium should show growth as evidenced by formation of colonies and/or a confluent lawn of growth. In the broth medium, growth is evident by the appearance of turbidity.

References

1. Lennox. 1955.Virology *1*:190.
2. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols in molecular biology, vol. 1. Green Publishing Associates, Inc., Brooklyn, N.Y.
3. Miller. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Availability

Difco™ LB Agar, Lennox

Cat. No. 240110 Dehydrated – 500 g

Difco™ LB Broth, Lennox

Cat. No. 240230 Dehydrated – 500 g
240210 Dehydrated – 2 kg
240220 Dehydrated – 10 kg

LB Agar, Miller • LB Broth, Miller

Intended Use
LB Agar, Miller and LB Broth, Miller (Luria-Bertani) are used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures.

Summary and Explanation
LB Agar, Miller and LB Broth, Miller are based on LB Medium as described by Miller for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures.[1-3] These are nutritionally rich media designed for growth of pure cultures of recombinant strains. *E. coli* grows more rapidly because they provide the cells with amino acids, nucleotide precursors, vitamins and other metabolites that the microorganism would otherwise have to synthesize.[4]

LB Broth, Miller contains twenty times the sodium chloride level of Luria Broth Base, Miller and twice the level found in LB Broth, Lennox.[3,5,6] This allows the researcher to select the optimal salt concentration for a specific strain in LB Agar, Miller.

Principles of the Procedure
Peptone provides nitrogen and carbon. Vitamins (including B vitamins) and certain trace elements are provided by yeast extract. Sodium ions for transport and osmotic balance are provided by sodium chloride. Agar is the solidifying agent in LB Agar, Miller.

User Quality Control

Identity Specifications
Difco™ LB Agar, Miller

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Very light amber, slightly opalescent.
Reaction of 4.0% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ LB Broth, Miller

Dehydrated Appearance:	Off-white to beige, free-flowing, homogeneous.
Solution:	2.5% solution; soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Very light amber, clear to very slightly opalescent.
Reaction of 2.5% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response
Difco™ LB Agar, Miller or LB Broth, Miller

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	33526 (K802)	10^2-10^3	Good

Formulae
Difco™ LB Agar, Miller

Approximate Formula* Per Liter

Tryptone	10.0	g
Yeast Extract	5.0	g
Sodium Chloride	10.0	g
Agar	15.0	g

Difco™ LB Broth, Miller

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ LB Agar, Miller – 40 g;
   Difco™ LB Broth, Miller – 25 g.
   Mix thoroughly.
2. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Consult appropriate references for recommended test procedures.[3-5]

Expected Results
Growth should be evident on the agar medium by the appearance of colonies and/or a confluent lawn on the surface of the medium. In the broth medium, growth is evident by the appearance of turbidity.

References
1. Luria and Burrous. 1955. J. Bacteriol. *74*:461.
2. Luria, Adams and Ting. 1960. Virology *12*:348.
3. Miller. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols in molecular biology, vol. 1. Greene Publishing Associates, Inc., Brooklyn, N.Y.
5. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd. ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. Lennox. 1955. Virology *1*:190.

Availability
Difco™ LB Agar, Miller

CCAM

Cat. No.	244520	Dehydrated – 500 g
	244510	Dehydrated – 2 kg

Difco™ LB Broth, Miller

Cat. No.	244620	Dehydrated – 500 g
	244610	Dehydrated – 2 kg
	214906	Dehydrated – 10 kg

Europe

Cat. No.	257269	Prepared Bottles, 450 mL – Pkg. of 10

LB Broth Base (Animal Free)

Intended Use

Select APS™ LB Broth Base is an animal-free medium used to grow and maintain recombinant strains of *Escherichia coli*.

Summary and Explanation

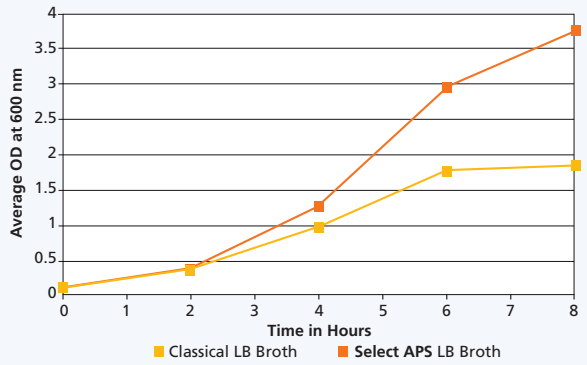
Select Alternative Protein Source (APS) media were designed as alternatives to classical animal-based media for the maintenance and propagation of *Escherichia coli* strains in molecular genetics procedures. **Select APS** media are manufactured from animal-free ingredients in order to minimize the risk of bovine spongiform encephalopathy in culture media containing animal, and especially bovine, materials. **Select APS** media are nutrient-rich formulations designed to out-perform classical animal-based molecular genetics media formulations.

Select APS LB Broth Base is based on the LB Broth Lennox formulation (1% tryptone, 0.5% yeast extract and 0.5% sodium chloride) with 5.0 g/L sodium chloride, which was developed by Lennox for the growth and maintenance of recombinant strains of *E. coli*.[1] The tryptone in the classical LB Lennox formulation is replaced by a combination of animal-free soy hydrolysate and yeast extract in the **Select APS** LB Broth Base.

Select APS LB Broth Base is an excellent all-purpose growth medium for the propagation and maintenance of *E. coli* in molecular biology procedures. Figure 1 shows *E. coli* DH5α growth curves comparing the classical LB Broth Base formulation to **Select APS** LB Broth Base in shaker flask culture. The **Select APS** LB Broth allowed for faster growth of the plasmid-carrying *E. coli* strain and showed twice the optical density (OD) after eight hours as did the classical LB Broth formulation containing tryptone.[2]

Figure 1. Growth Curve

Growth Performance of Classical LB Broth Base vs. **Select APS**™ LB Broth *E. coli* DH5α



Principles of the Procedure

Soy hydrolysate provides nitrogen and carbon essential for bacterial metabolism. Yeast extract supplies vitamins, amino acids and trace elements which enhance bacterial growth and plasmid yield. Sodium chloride provides sodium ions for transport and osmotic balance.

Formula

BBL™ Select APS™ LB Broth Base

Approximate Formula* Per Liter		
Soy Hydrolysate	2.5	g
Yeast Extract	12.5	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 20.0 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for details on recommended test procedures.[3,4]

Expected Results

Growth is evident by the appearance of turbidity.

References

1. Lennox. 1955. Virology. *1*:190.
2. BD Biosciences. 2006. BD Bionutrients™ Technical Manual, Advanced Bioprocessing, 3rd ed. Becton, Dickinson, and Company, Sparks, Md.
3. Sambrook and Russell. 2001. Molecular cloning, a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
4. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl. 2002. Short protocols in molecular biology, 5th ed. John Wiley & Sons, Inc., Hoboken, N.J.

Availability

BBL™ Select APS™ LB Broth Base

Cat. No.		
	292438	Dehydrated – 500 g
	212484	Dehydrated – 10 kg

User Quality Control

Identity Specifications

BBL™ Select APS™ LB Broth Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water. Solution is light to medium, yellow to tan, slightly hazy to hazy.
Prepared Appearance:	Light to medium, yellow to tan, slightly hazy to hazy.
Reaction of 3.5% Solution at 25°C:	pH 6.6 – 7.1

Cultural Response

BBL™ Select APS™ LB Broth Base

Prepare the medium per label directions. Inoculate and incubate at 37°C, 250 rpm for 16 hours.

ORGANISM	ATCC™	RECOVERY
Escherichia coli	700790	Good

LBS Agar • LBS Broth

Intended Use

LBS (*Lactobacillus* Selection) Agar is used for the selective isolation and enumeration of lactobacilli.

LBS Broth is a selective medium for the isolation and cultivation of lactobacilli.

Summary and Explanation

Rogosa et al. developed LBS Agar as a selective medium for the isolation and enumeration of oral and fecal lactobacilli.[1,2] They reported that the medium was more selective for lactobacilli than the tomato juice media previously employed and that the growth of molds, streptococci and spreading organisms was markedly reduced.

LBS Agar is used for selective recovery of lactobacilli from the oral cavity, especially tooth surfaces, intestinal flora,[3,4] the vagina,[5] meats and other foods[6] and dairy products.[7] Sabine and Vaselekos reported that incorporation of tomato juice resulted in a two-fold increase in the number of *L. acidophilus* organisms recovered from feces.[8]

LBS Broth is the fluid form of LBS Agar.[1] This broth medium may be used as a preliminary enrichment broth for detection of lactic acid organisms. Subcultures should be made to LBS Agar or to suitable general-purpose plating media.

Principles of the Procedure

Peptone, yeast extract and dextrose supply nitrogenous and carbonaceous compounds for the support of bacterial growth. Polysorbate 80 is a source of growth factors, since it supplies fatty acids required for the metabolism of lactobacilli. The ammonium citrate and sodium acetate inhibit the growth of many organisms, including streptococci, molds and members of the oral microbial flora other than lactobacilli, and restrict swarming on the agar medium.[9] The low pH, due to the addition of acetic acid, also is inhibitory for other bacterial flora and favors the isolation of lactobacilli.

Formulae

BBL™ LBS Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Yeast Extract	5.0	g
Monopotassium Phosphate	6.0	g
Ammonium Citrate	2.0	g
Dextrose	20.0	g
Polysorbate 80	1.0	g
Sodium Acetate Hydrate	25.0	g
Magnesium Sulfate	575.0	mg
Manganese Sulfate	0.12	g
Ferrous Sulfate	34.0	mg
Agar	15.0	g

BBL™ LBS Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   BBL™ LBS Agar – 84 g (for growth of *L. acidophilus*, add 84 g to 200 mL of filtered tomato juice plus 800 mL of purified water);
   BBL™ LBS Broth – 70 g.
   Mix thoroughly.
2. Add 1.32 mL of glacial acetic acid (ACS).
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Cool and use without autoclaving. If storage is necessary, autoclave at 118°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

BBL™ LBS Agar

Dehydrated Appearance:	Fine, slightly clumped and moist, homogeneous, free of extraneous material.
Solution:	8.4% solution, soluble in purified water upon boiling. Solution (with acetic acid) is light to medium, yellow to tan, moderately hazy to hazy.
Prepared Appearance:	Light to medium, yellow to tan, moderately hazy.
Reaction of 8.4% Solution at 25°C:	pH 5.5 ± 0.2

BBL™ LBS Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	7.0% solution, soluble in purified water upon boiling. Solution (with acetic acid) is light to medium, yellow to tan, slightly hazy to clear.
Prepared Appearance:	Light to medium, yellow to tan, slightly hazy to clear.
Reaction of 7.0% Solution at 25°C:	pH 5.4 ± 0.2

Cultural Response

BBL™ LBS Agar or LBS Broth

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C with 3-5% CO_2 for 42-48 hours (up to 4 days, if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus acidophilus	4356	Undiluted	Good
Lactobacillus plantarum	8014	Undiluted	Good
Proteus vulgaris	8427	10^4-10^5	Partial to complete inhibition

Procedure

LBS Agar can be used as a plating medium for counting lactobacilli in various types of specimens. Alternatively, it can be used for direct recovery of organisms using the streak-inoculation technique. Incubate plates at 35 ± 2°C in an aerobic or anaerobic atmosphere supplemented with carbon dioxide.

Inoculate the broth with the test specimen and incubate tubes with loosened caps at 35 ± 2°C in an aerobic or anaerobic atmosphere supplemented with carbon dioxide.

Expected Results

On the agar medium, lactobacilli appear as large, white colonies. Growth should be subcultured to appropriate agar or broth media for use in biochemical identification procedures.

After growth has been obtained in LBS Broth, as evidenced by the appearance of turbidity in the tube, subculture aliquots to appropriate plating media (e.g., LBS Agar) in order to obtain isolates in pure culture for subsequent use in identification procedures.

Limitations of the Procedure

1. LBS Agar should not be used for the maintenance of lactobacilli, since it is not sterile and, being a highly selective medium, is not favorable for keeping cultures in their normal state. Colonies required for further study should be subcultured as soon as possible.
2. This medium is not suitable for the isolation of dairy lactobacilli such as *L. lactis* and *L. bulgaricus* due to its content of salts.[9]

References

1. Rogosa, Mitchell and Wiseman. 1951. J. Bacteriol. *62*:132.
2. Rogosa, Mitchell and Wiseman. 1951. J. Dental Res. *30*:682.
3. Wiseman, Sarles, Benton, Harper and Elvehjem. 1956. J. Bacteriol. *79*:723.
4. Ellis and Sarles. 1958. J. Bacteriol. *75*:272.
5. Rogosa and Sharpe. 1960. J. Gen. Microbiol. *23*:197.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
8. Sabine and Vaselekos. 1965. Nature *206*:960.
9. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ LBS Agar

COMPF SMD

Cat. No. 211327 Dehydrated – 500 g*

BBL™ LBS Broth

Cat. No. 211331 Dehydrated – 500 g*

**Store at 2-8°C.*

LPM Agar Base
Listeria Selective Supplement

Intended Use

LPM Agar Base is used with Listeria Selective Supplement for isolating and cultivating *Listeria monocytogenes*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported foodborne outbreak of listeriosis was in 1985,[3] and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *L. monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté, and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[6]

Listeria species grow over a pH range of 4.4-9.6, and survive in food products with pH levels outside these parameters.[7] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

LPM Agar, a modification of McBride Listeria Agar, was developed by Lee and McClain[9] to recover low numbers of *L. monocytogenes* from samples with profusely mixed microflora. Its use is recommended when testing food and dairy samples and clinical specimens for *Listeria*.

User Quality Control

Identity Specifications

Difco™ LPM Agar Base

Dehydrated Appearance:	Light tan, homogeneous, may have a tendency to clump.
Solution:	5.05% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 5.05% Solution at 25°C:	pH 7.3 ± 0.2

BBL™ Listeria Selective Supplement

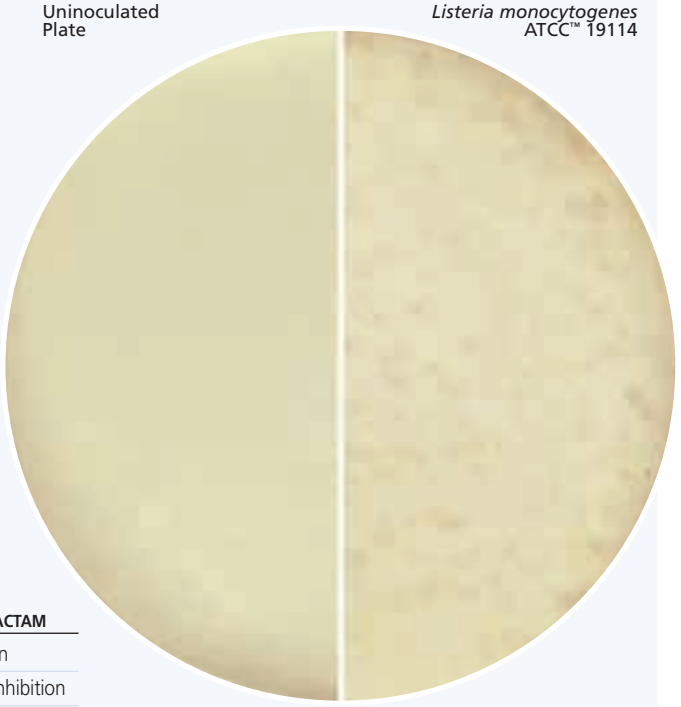
Lyophilized Appearance:	White to pale yellow and dry.
Rehydrated Appearance:	Clear to pale yellow to light tan yellow; clear.

Cultural Response

Difco™ LPM Agar Base

Prepare the medium per label directions with Listeria Selective Supplement. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	GROWTH W/MOXALACTAM
Bacillus subtilis	6633	10^3	Partial inhibition
Enterococcus faecalis	29212	10^3-2×10^3	Marked to complete inhibition
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition
Listeria monocytogenes	19114	10^2-10^3	Good (at 40-48 hours)
Staphylococcus aureus	25923	10^3	Marked to complete inhibition



Principles of the Procedure

In LPM Agar, peptones and beef extract provide nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. Glycine anhydride is used for improved recovery of *Listeria*. Lithium chloride, in an increased concentration, and phenylethanol are incorporated to aid in suppression of both gram-positive and gram-negative contaminants. Agar is the solidifying agent. Listeria Selective Supplement is added to LPM Agar Base after autoclaving to inhibit staphylococci, bacilli and *Proteus* species.

Formulae

Difco™ LPM Agar Base

Approximate Formula* Per Liter

Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	5.0	g
Beef Extract	3.0	g
Sodium Chloride	5.0	g
Lithium Chloride	5.0	g
Glycine Anhydride	10.0	g
Phenylethanol	2.5	g
Agar	15.0	g

BBL™ Listeria Selective Supplement

Formula Per 1 mL Vial

Moxalactam	0.01	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ LPM Agar Base

1. Suspend 50.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45-50°C.
5. Aseptically add 2.0 mL of the reconstituted Listeria Selective Supplement to 1 L of medium. Mix well.
6. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Listeria Selective Supplement

1. Reconstitute each lyophilized vial by aseptically adding 2.0 mL of sterile purified water with a sterile syringe and needle.
2. Invert the vial several times to assure complete solution.

Procedure

Clinical specimens obtained from nonsterile sites should be selectively enriched for *Listeria* spp. before being plated. Refer to appropriate references for the procedure to use with clinical specimens.[10] For a procedure for isolating *Listeria* from milk, milk products and food samples, refer to an appropriate reference.[7,11,12]

Expected Results

Observe colonies under oblique transmitted light. *Listeria* colonies display a gray to blue color with a ground glass appearance.

References

1. Murray, Webb and Swann. 1926. J. Pathol. Bacteriol. *29*:407.
2. Monk, Clavero, Beuchat, Doyle and Brackett. 1994. J. Food Prot. *57*:969.
3. Wehr. 1987. J. Assoc. Off. Anal. Chem. *70*:769.
4. Bremer and Osborne. 1995. J. Food Prot. *58*:604.
5. Grau and Vanderlinde. 1992. J. Food Prot. *55*:4.
6. Patel, Hwang, Beuchat, Doyle and Brackett. 1995. J. Food Prot. *58*:244.
7. Ryser and Donnelly. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
8. Kramer and Jones. 1969. J. Appl. Bacteriol. *32*:381.
9. Lee and McClain. 1986. Appl. Environ. Microbiol. *52*:1215.
10. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
11. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
12. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ LPM Agar Base

AOAC BAM CCAM COMPF SMD

Cat. No.	222120	Dehydrated – 500 g*
	222110	Dehydrated – 2 kg*

BBL™ Listeria Selective Supplement

AOAC BAM CCAM COMPF SMD

Cat. No.	212402	Vial – 10 × 2 mL*

**Store at 2-8°C.*

Lactobacilli Agar AOAC • Lactobacilli Broth AOAC

Intended Use

Lactobacilli Agar AOAC is used for maintaining stock cultures for microbiological assays of vitamins and amino acids.

Lactobacilli Broth AOAC is used for preparing inocula for microbiological assays of vitamins and amino acids.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For maintaining the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Lactobacilli Agar AOAC[1] and Lactobacilli Broth AOAC[1] are prepared according to the formula recommended by Loy.[2] Lactobacilli Agar AOAC is used for maintaining stock cultures. Lactobacilli Broth AOAC is used to prepare inocula of *Lactobacillus delbruecki* subsp. *lactis (Lactobacillus leichmannii)* ATCC™ 7830, *Enterococcus hirae* ATCC 8043, *Lactobacillus plantarum* ATCC 8014, *Lactobacillus (casei) rhamnosus* ATCC 7469 and other organisms used in the microbiological assay of B vitamins.

Lactobacillus species grow poorly on nonselective culture media and require special nutrients. Mickle and Breed[3] reported the use of tomato juice in culture media for lactobacilli. Kulp and White,[4] while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.

User Quality Control

Identity Specifications

Difco™ Lactobacilli Agar AOAC

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	4.8% solution, soluble in purified water upon boiling 2-3 minutes. Solution is medium amber, opalescent when hot, clearer when cooled to 45-50°C.
Prepared Appearance:	Medium amber, clear.
Reaction of 4.8% Solution at 25°C:	pH 6.8 ± 0.2

Difco™ Lactobacilli Broth AOAC

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water upon boiling 2-3 minutes. Solution is medium amber, opalescent when hot, clear after cooling, may have a slight precipitate.
Prepared Appearance:	Medium amber, clear, may have a slight precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Lactobacilli Agar AOAC or Lactobacilli Broth AOAC

Prepare the medium per label directions. Inoculate Lactobacilli Agar AOAC by stabbing the medium with test organisms; incubate at 35 ± 2°C for 18-48 hours. Inoculate Lactobacilli Broth AOAC with test organisms and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus hirae	8043	10^2-10^3	Good
Lactobacillus rhamnosus	7469	10^2-10^3	Good
Lactobacillus delbrueckii subsp. *lactis*	7830	10^2-10^3	Good
Lactobacillus plantarum	8014	10^2-10^3	Good

Principles of the Procedure

Peptonized milk and yeast extract provide the nitrogen, amino acids and vitamins sources in Lactobacilli Agar AOAC and Lactobacilli Broth AOAC. Dextrose is a carbon source to facilitate organism growth. Tomato juice creates the proper acidic environment. Monopotassium phosphate is a buffering agent. Polysorbate 80 acts as an emulsifier. Agar is the solidifying agent in Lactobacilli Agar AOAC.

Formulae

Difco™ Lactobacilli Agar AOAC

Approximate Formula* Per Liter

Peptonized Milk	15.0	g
Yeast Extract	5.0	g
Dextrose	10.0	g
Tomato Juice (from 100 mL)	5.0	g
Monopotassium Phosphate	2.0	g
Polysorbate 80	1.0	g
Agar	10.0	g

Difco™ Lactobacilli Broth AOAC

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware used for microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemical must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform.

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Lactobacilli Agar AOAC – 48 g;
   Difco™ Lactobacilli Broth AOAC – 38 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Stock Cultures

1. Prepare stock cultures in one or more tubes of sterile Lactobacilli Agar AOAC.
2. Inoculate the medium using an inoculating needle.
3. Incubate at 30-37°C for 18-24 hours.
4. Store at 2-8°C.
5. Transfer at weekly or twice monthly intervals.

Inoculum

1. Subculture from a 16-24 hour stock culture into 10 mL Lactobacilli Broth AOAC.
2. Incubate at 35-37°C for 16-24 hours or as specified in specific assay procedures.
3. Centrifuge the culture and decant the supernatant.
4. Resuspend cells in 10 mL of sterile 0.9% NaCl solution or sterile single-strength basal assay medium.
5. Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise indicated.
6. Dilute the washed suspension 1:100 with sterile 0.9% NaCl or sterile single-strength basal assay medium or as indicated. Where applicable, inoculum concentration should be adjusted according to limits specified in the references.[1,5]

For a complete discussion on vitamin assay methodology refer to appropriate procedures outlined in the references.[1,5]

Expected Results

Refer to appropriate references for vitamin assay results.[1,5]

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for that purpose.
2. Aseptic technique should be used throughout the vitamin assay procedure.
3. The use of altered or deficient media may result in mutants with different nutritional requirements that will not give a satisfactory response.
4. For a successful completion of these procedures, all conditions of the assay must be adhered to meticulously.

References

1. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
2. Loy. 1958. J. Assoc. Off. Agri. Chem. *4*:61.
3. Mickle and Breed. 1925. Technical Bulletin 110, N.Y. State Agriculture Ex. Station, Geneva, N.Y.
4. Kulp and White. 1932. Science 76:17.
5. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26 Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Lactobacilli Agar AOAC

AOAC

Cat. No. 290010 Dehydrated – 100 g*

Difco™ Lactobacilli Broth AOAC

AOAC

Cat. No. 290110 Dehydrated – 100 g*

**Store at 2-8°C.*

Lactobacilli MRS Agar • Lactobacilli MRS Broth

Intended Use

Lactobacilli MRS Agar and Lactobacilli MRS Broth are recommended for use in the isolation, enumeration and cultivation of *Lactobacillus* species.

Summary and Explanation

Lactobacilli MRS Agar and Lactobacilli MRS Broth are based on the formulations of deMan, Rogosa and Sharpe.[1] These media were shown by the authors to support luxuriant growth of all lactobacilli from oral, fecal, dairy and other sources.

Principles of the Procedure

Lactobacilli MRS Agar and Lactobacilli MRS Broth contain peptone and dextrose. These ingredients supply nitrogen, carbon and other elements necessary for growth. Polysorbate 80, acetate, magnesium and manganese provide growth factors for culturing a variety of lactobacilli. The above ingredients may inhibit the growth of some organisms other than lactobacilli.

Formulae

Difco™ Lactobacilli MRS Agar

Approximate Formula* Per Liter

Proteose Peptone No. 3	10.0	g
Beef Extract	10.0	g
Yeast Extract	5.0	g
Dextrose	20.0	g
Polysorbate 80	1.0	g
Ammonium Citrate	2.0	g
Sodium Acetate	5.0	g
Magnesium Sulfate	0.1	g
Manganese Sulfate	0.05	g
Dipotassium Phosphate	2.0	g
Agar	15.0	g

Difco™ Lactobacilli MRS Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water.
   Difco™ Lactobacilli MRS Agar – 70 g;
   Difco™ Lactobacilli MRS Broth – 55 g.
   Mix thoroughly.

User Quality Control

Identity Specifications

Difco™ Lactobacilli MRS Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	7.0% solution, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent.
Prepared Appearance:	Medium amber, very slightly to slightly opalescent.
Reaction of 7.0% Solution at 25°C:	pH 6.5 ± 0.2

Difco™ Lactobacilli MRS Broth

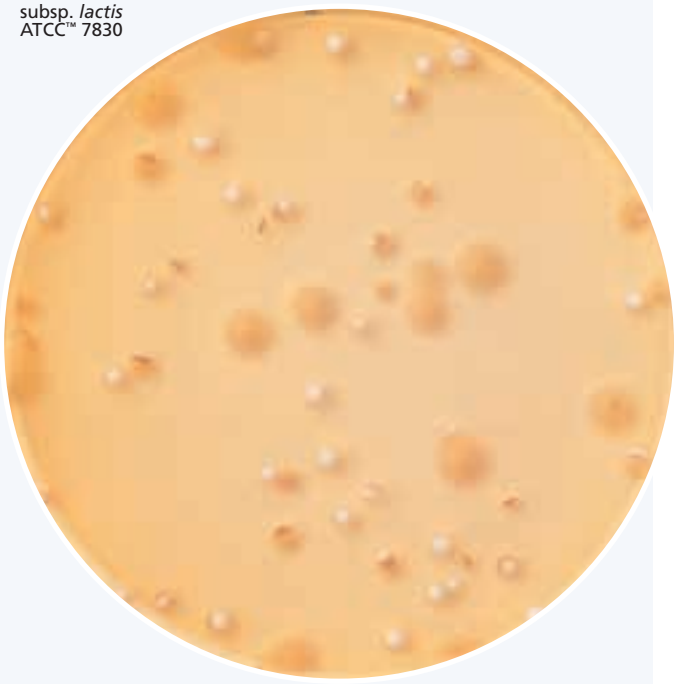
Dehydrated Appearance:	Tan, homogeneous, appears moist.
Solution:	5.5% solution, soluble in purified water upon boiling. Solution is medium amber, clear to very slightly opalescent.
Prepared Appearance:	Medium amber, clear to very slightly opalescent.
Reaction of 5.5% Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

Difco™ Lactobacilli MRS Agar or Lactobacilli MRS Broth

Prepare the medium per label directions. Inoculate Lactobacilli MRS Agar and incubate in a 5% CO_2 atmosphere at 35° ± 2°C for 24- 72 hours. Inoculate Lactobacilli MRS Broth and incubate at 35° ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus delbrueckii subsp. *lactis*	7830	10^2-10^3	Good
Lactobacillus fermentum	9338	10^2-10^3	Good
Lactobacillus johnsonii	11506	10^2-10^3	Good



Lactobacillus delbrueckii subsp. *lactis* ATCC™ 7830

2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Direct Counts

1. To obtain direct counts of lactobacilli, pour 15-20 mL sterile, molten (45-50°C) Lactobacilli MRS Agar into sterile Petri dishes containing 1 mL volumes of diluted test sample.
2. Distribute the inoculum throughout the medium by rotating the plate in one direction and then in the reverse direction.
3. Allow the medium to solidify on a flat surface for 5-10 minutes.
4. Alternatively, plates of Lactobacilli MRS Agar can be used for direct recovery of organisms using the streak inoculation technique.
5. Incubate agar plates at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere supplemented with carbon dioxide.

Broth Enrichment

1. Samples can be inoculated directly into Lactobacilli MRS Broth.
2. Incubate broth tubes at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere.
3. Subculture growth in broth tubes to appropriate solid media.

Expected Results

Lactobacilli appear as large, white colonies embedded in or on Lactobacilli MRS Agar or as turbidity in Lactobacilli MRS Broth. Growth may be subcultured onto the appropriate media for use in additional procedures. Refer to appropriate references for recommendations on the culture of *Lactobacillus* spp.[2,3]

Limitation of the Procedure

Organisms other than lactobacilli may grow in these media. Isolates must be confirmed as lactobacilli by appropriate biochemical testing.

References

1. deMan, Rogosa and Sharpe. 1960. J. Appl. Bacteriol. *23*:130.
2. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Downes and Ito(ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Lactobacilli MRS Agar

COMPF ISO SMD

Cat. No.	288210	Dehydrated – 500 g*

Difco™ Lactobacilli MRS Broth

COMPF SMD

Cat. No.	288130	Dehydrated – 500 g*
	288110	Dehydrated – 2 kg*
	288120	Dehydrated – 10 kg*

**Store at 2-8°C.*

Lactose Broth

Intended Use

Lactose Broth is used for detection of the presence of coliform organisms, as a pre-enrichment broth for salmonellae and in the study of lactose fermentation of bacteria in general.

Summary and Explanation

Lactose Broth was formulated in accordance with recommendations of the American Public Health Association (APHA) and the American Water Works Association for testing dairy products and water for the presence of coliform organisms.[1,2] This medium was, but no longer is, listed as an alternative to Lauryl Sulfate Broth in the presumptive portion of the Standard Total Coliform Multiple-Tube (MPN) Test for water analysis provided that it had been demonstrated not to increase the frequency of false-positives nor mask coliforms.[3] It is one of the recommended media in the *Compendium of Methods for the Microbiological Examination of Foods* for pre-enrichment when *Salmonella* organisms are suspected in foods.[4]

Principles of the Procedure

The peptone and beef extract provide essential nutrients for bacterial metabolism. Lactose provides a source of fermentable carbohydrate for coliform organisms. Growth with the formation of gas is a presumptive test for coliforms.

Formula

Difco™ Lactose Broth

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	5.0	g
Lactose	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 13 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm gently until solution is complete.
3. Dispense in test tubes containing inverted Durham tubes, in 10 mL amounts for testing samples of 1 mL or less. For testing 10 mL quantities of samples, dissolve 26 g of the powder in

User Quality Control

Identity Specifications

Difco™ Lactose Broth

Dehydrated Appearance:	Light beige to light tan, free flowing, homogeneous.
Solution:	1.3% solution, soluble in purified water upon slight warming. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 1.3% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Lactose Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. After incubation, add 1-2 drops of 1% phenol red solution to observe acid production.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID	GAS
Enterobacter aerogenes	13048	30-300	Good	+	+
Escherichia coli	25922	30-300	Good	+	+
Enterococcus faecalis	19433	30-300	Good	+	–
Salmonella enterica subsp. *enterica* serotype Typhi	6539	30-300	Good	–	–

Uninoculated Tube

Escherichia coli ATCC™ 25922

1 L of purified water and distribute in 10 mL amounts. The concentration of the medium should be varied according to the size of the test samples. The concentration of broth plus sample should approximate 1× for proper performance. (In broth concentrations higher than 2×, a reduction in clarity may be noticed.)

4. Autoclave at 121°C for 15 minutes.
5. After autoclaving, cool the broth as quickly as possible.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Refer to the official test procedures for the detection of coliforms in the compendia of methods for the microbiological examination of foods.[4]

Expected Results

After incubation at 35 ± 2°C for 24 ± 2 hours, examine tubes for turbidity and for gas production in the Durham tubes. If no gas has formed and been trapped in the inverted tube, reincubate and reexamine after 48 ± 3 hours.

Turbidity of the medium accompanied by formation of gas in any amount in the Durham tubes within 48 ± 3 hours is a positive presumptive test for the presence of coliforms in the sample. The result should be confirmed by additional standard testing.

References

1. American Public Health Association. 1946. Standard methods for the examination of water and sewage, 9th ed. APHA, New York, N.Y.
2. American Public Health Association. 1948. Standard methods for the examination of dairy products, 9th ed. APHA, New York, N.Y.
3. Rand, Greenberg and Taras (ed.). 1976. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
4. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Lactose Broth

AOAC BAM CCAM COMPF SMD

Cat. No.	243000	Dehydrated – 100 g
	211835	Dehydrated – 500 g
	241000	Dehydrated – 2 kg
	242000	Dehydrated – 10 kg
	290701	Prepared Bottles, 90 mL (Wide Mouth) – Pkg. of 10*

Europe

Cat. No.	256668	Prepared Bottles, 90 mL – Pkg. of 10*

**Store at 2-8°C.*

L

Lactose Peptone Broth

Intended Use

Lactose Peptone Broth is used for the detection of coliform organisms in water.

Summary and Explanation

Lactose Peptone Broth is based on the Lactose Peptone Broth formula described in German Standard Methods and German Drinking Water Regulations.[1] Lactose Peptone Broth is recommended as a nonselective broth enrichment and detection medium for *E. coli* and other coliform bacteria present in water. Lactose fermentation and gas production at 36 ± 1°C are used as the basis for this presumptive coliform test.

Principles of the Procedure

Lactose Peptone Broth contains peptones, which provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Lactose is provided as a source of fermentable carbohydrate. Sodium chloride is present in the medium to provide a suitable osmotic environment. Bromcresol purple is used as a colorimetric indicator to show the production of acid from the fermentation of lactose.

Formula

Difco™ Lactose Peptone Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	17.0	g
Soy Peptone	3.0	g
Lactose	10.0	g
Sodium Chloride	5.0	g
Bromcresol Purple	0.02	g

**Adjusted and/or supplemented ad required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 35 g (single strength) or 105 g (triple strength) of the powder, depending upon the test procedure, in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense 50 mL into tubes or bottles containing a Durham tube.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure[1]

Direct Broth Method

1. Add 100 mL of sample to 50 mL of triple strength Lactose Peptone Broth.
2. Incubate at 36 ± 1°C for 24-48 hours.
3. Examine tubes or bottles for evidence of acid formation and gas production.

Membrane Filtration Broth Method

1. Filter 100 mL of sample through a sterile 0.45 micron membrane filter.
2. Remove filter and place in 50 mL of single strength Lactose Peptone Broth.
3. Incubate at 36 ± 1°C for 24-48 hours.
4. Examine tubes or bottles for evidence of acid formation and gas production.

User Quality Control

Identity Specifications

Difco™ Lactose Peptone Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% (single strength) and 10.5% (triple-strength) solution, soluble in purified water. Solution is dark reddish-purple, clear to slightly opalescent.
Prepared Appearance:	Dark reddish-purple, clear to slightly opalescent.
Reaction of 10.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Lactose Peptone Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	ACID	GAS
Escherichia coli	25922	$10\text{-}10^2$	+	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	$10^2\text{-}10^3$	–	–

Expected Results

Acid formation is demonstrated by a change in the color of the medium from reddish-purple to yellow. Gas production is demonstrated by the displacement of the medium from the Durham tube. Production of both acid and gas is a presumptive indication of the presence of coliform organisms.

Subculture presumptive positives onto Endo Agar and MacConkey Agar. Incubate at 35 ± 2°C for 24 hours. Examine plates for the presence of typical coliform colonies. Further biochemical testing is necessary to confirm the presence and identify coliforms. Consult appropriate references for further information on identification of coliforms.[2,3]

Limitation of the Procedure

Detection of coliform bacteria in Lactose Peptone Broth using this method is only a presumptive test.

References

1. DIN Deutsches Institut für Normung. 1991. e.V.: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammunter suchung: Mikrobiologische Verfahren (Gruppe k), Nachwels von *Escherichia coli* und coliformen Keimen (K6). Reference Method DIN 38411.
2. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Lactose Peptone Broth

Cat. No.	266520	Dehydrated – 500 g
	266510	Dehydrated – 10 kg

Lauryl Tryptose Broth • Lauryl Sulfate Broth

Intended Use

Lauryl Tryptose Broth and Lauryl Sulfate Broth, which are also known as Lauryl Sulfate Tryptose (LST) Broth, are used for the detection of coliform organisms in materials of sanitary importance.

Summary and Explanation

Mallmann and Darby developed this medium for the detection of coliform organisms by American Public Health Association (APHA) procedures.[1] They incorporated sodium lauryl sulfate into the formulation since it proved to be selective but not inhibitory for coliforms.

This medium is used for the detection of coliforms in foods [2] and dairy products.[3] It is now the medium of choice for use in the presumptive phase of the Standard Total Coliform Multiple-Tube (MPN) Test for the microbiological examination of water.[4] It is also listed in the *Official Methods of Analysis of AOAC International*.[5]

Principles of the Procedure

Peptone provides essential growth substances, such as nitrogen and carbon compounds, sulfur and trace ingredients. The potassium phosphates provide buffering capacity. Sodium chloride maintains osmotic equilibrium.

Lactose provides a source of fermentable carbohydrate for coliform organisms. The fermentation of lactose with gas formation is a presumptive test for coliforms. Sodium lauryl sulfate inhibits organisms other than coliforms.

Formulae

Difco™ Lauryl Tryptose Broth

Approximate Formula* Per Liter

Tryptose	20.0	g
Lactose	5.0	g
Dipotassium Phosphate	2.75	g
Monopotassium Phosphate	2.75	g
Sodium Chloride	5.0	g
Sodium Lauryl Sulfate	0.1	g

BBL™ Lauryl Sulfate Broth

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0	g
Lactose	5.0	g
Dipotassium Phosphate	2.75	g
Monopotassium Phosphate	2.75	g
Sodium Chloride	5.0	g
Sodium Lauryl Sulfate	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Lauryl Tryptose Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.56% solution, soluble in purified water upon warming. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 3.56% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Lauryl Tryptose Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 ± 2 hours or longer, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS
Enterobacter aerogenes	13048	30-300	Good	+*
Escherichia coli	25922	30-300	Good	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	–
Staphylococcus aureus	25923	3×10^2-10^3	Marked to complete inhibition	–

**Gas production positive within 48 ± 3 hours.*

Identity Specifications

BBL™ Lauryl Sulfate Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.56% solution, soluble in purified water. Solution is pale to light, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Pale to light, tan to yellow, clear to slightly hazy.
Reaction of 3.56% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Lauryl Sulfate Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS
Enterobacter aerogenes	13048	10^3-10^4	Good	+
Enterococcus faecalis	29212	10^4-10^5	Partial to complete inhibition	–
Escherichia coli	25922	10^3-10^4	Good	+
Proteus mirabilis	12453	10^3-10^4	Good	–

Directions for Preparation from Dehydrated Product

Difco™ Lauryl Tryptose Broth

1. Suspend 35.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense required amounts into tubes containing inverted fermentation vials (see table).
4. Autoclave at 121°C for 15 minutes. Cool the broth as quickly as possible.
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Lauryl Sulfate Broth

1. Suspend 35.6 g of the powder in 1 L of purified water.
2. Dispense in test tubes, containing inverted Durham tubes, in 10 mL amounts for testing samples of 1 mL or less. For testing 10 mL quantities of samples, dissolve 71.2 g of the powder in 1 L of purified water and distribute in 10 mL amounts. The concentration of the medium should be varied according to the size of the test samples (see table).
3. Autoclave at 121°C for 15 minutes. After autoclaving, cool the broth as quickly as possible.
4. Test samples of the finished product for performance using stable, typical control cultures.

Preparation of Lauryl Tryptose (Sulfate) Broth[4]

INOCULUM mL	AMOUNT OF MEDIUM IN THE TUBE mL	VOLUME OF MEDIUM+INOCULUM mL	DEHYDRATED MEDIUM REQUIRED g/L
1	10 or more	11 or more	35.6
10	10	20	71.2
10	20	30	53.4
20	10	30	106.8
100	50	150	106.8
100	35	135	137.1
100	20	120	213.6

NOTE: Refrigerated broth generally becomes cloudy or forms precipitates but clears upon warming to room temperature. However, clarity is not important because only gas production is significant.

Procedure

Refer to the official test procedures for the detection of coliforms in the compendia of methods for microbiological examination of foods, dairy products and waters.[2-5]

Expected Results

After incubation of the tubes with loosened caps at 35 ± 0.5°C for 24 hours, examine for turbidity and for gas production in the Durham fermentation tubes. If no gas has formed and been trapped in the inverted tube, reincubate and reexamine after 48 hours.[2-5]

Turbidity of the medium accompanied by formation of gas in any amount in the Durham tubes within 48 hours is a positive presumptive test for the presence of coliforms in the sample.[2-5] The result should be confirmed by additional standard testing.

References

1. Mallmann and Darby. 1941. Am. J. Public Health *31*:127.
2. Downes and Ito. 2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, Washington, D.C.
3. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association. Washington, D.C.
4. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
5. Horwitz (ed.). 2007. Official methods of analysis of AOAC International. 18th ed., online. AOAC International. Gaithersburg, Md.

Availability

Difco™ Lauryl Tryptose Broth

AOAC BAM CCAM COMPF ISO SMD SMWW

Cat. No.	224140	Dehydrated – 100 g
	224150	Dehydrated – 500 g
	224120	Dehydrated – 2 kg
	224130	Dehydrated – 10 kg

BBL™ Lauryl Sulfate Broth

AOAC BAM CCAM COMPF EPA ISO SMD SMWW

Cat. No.	211338	Dehydrated – 500 g
	211339	Dehydrated – 5 lb (2.3 kg)
	294369	Dehydrated – 25 lb (11.3 kg)

Lauryl Tryptose Broth with MUG
Lauryl Sulfate Broth with MUG

Intended Use

Lauryl Tryptose Broth with MUG and Lauryl Sulfate Broth with MUG, which are also known as Lauryl Sulfate Tryptose Broth with MUG (LST-MUG), are used for the detection of *Escherichia coli* in water, food and dairy samples by a fluorogenic procedure.

Summary and Explanation

E. coli is used as an indicator organism of unsanitary conditions. A number of selective media are recommended for use in enrichment, presumptive identification and confirmatory procedures for demonstrating the presence of coliforms in material of sanitary importance. These procedures require the incubation of test media for up to 7 days.

The presence of the fluorogenic compound, MUG (4-methylumbelliferyl-β-D-glucuronide), in this medium permits the rapid detection of *E. coli* when the medium is observed for fluorescence using a long-wave UV light source, and further confirmation is not required.[1,2] MUG detects anaerogenic strains which may not be detected in the conventional procedure.[1]

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Lauryl Tryptose Broth with MUG

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	3.57% solution, soluble in purified water upon warming. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 3.57% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Lauryl Tryptose Broth with MUG

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 ± 2 hours or longer, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS	FLUORESCENCE
Enterobacter aerogenes	13048	30-100	Good	+*	–
Escherichia coli	25922	30-100	Good	+	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-100	Good	–	–
Staphylococcus aureus	25923	3×10^2-10^3	Marked to complete inhibition	–	–

**Gas production positive within 48 ± 3 hours.*

Identity Specifications

BBL™ Lauryl Sulfate Broth with MUG

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.57% solution, soluble in purified water. Solution is pale to light, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Pale to light, tan to yellow, clear to slightly hazy.
Reaction of 3.57% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Lauryl Sulfate Broth with MUG

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS	FLUORESCENCE
Enterobacter aerogenes	13048	10^3-10^4	Good	+	–
Enterococcus faecalis	29212	10^4-10^5	Partial to complete inhibition	–	–
Escherichia coli	25922	10^3-10^4	Good	+	+

Feng and Hartman, using a MUG-containing medium in microtitration plates, reported β-glucuronidase activity in 96% of *E. coli*, 100% of enterotoxigenic *E. coli*, 17% of *Salmonella* spp., and 40% of *Shigella* spp., while all other genera tested were negative; most reactions occurred within 4 hours, but some weakly β-glucuronidase-positive strains required overnight incubation.[1] In the presence of large numbers of *Proteus vulgaris*, which may suppress gas production by *E. coli*, fluorescence due to *E. coli* was detected within 15 hours.[1]

In a comparison, with conventional methods, Robison reported 94.8% agreement, a false-positive rate of 4.8%, attributable to the presence of streptococci in the samples, and no false-negatives.[2]

These media are included in the compendia of methods for the detection and enumeration of coliform organisms in food [3] and dairy [4] products and in the *Official Methods of Analysis of AOAC International*.[5]

Principles of the Procedure

Lactose is a source of energy for organisms. Peptone provides additional nutrients. The phosphate compounds provide buffering capacity. Sodium lauryl sulfate is inhibitory to many organisms but not for coliforms.

The substrate 4-methylumbelliferyl-β-D-glucuronide is hydrolyzed by an enzyme, β-glucuronidase, possessed by most *E. coli* and a few strains of *Salmonella*, *Shigella* and *Yersinia*, to yield a fluorescent end product, 4-methylumbelliferone.[1,2] Development of fluorescence permits the detection of *E. coli* in pure or mixed cultures within 4-24 hours following inoculation and incubation of the medium.

Formulae

Difco™ Lauryl Tryptose Broth with MUG

Approximate Formula* Per Liter		
Tryptose	20.0	g
Lactose	5.0	g
Dipotassium Phosphate	2.75	g
Monopotassium Phosphate	2.75	g
Sodium Chloride	5.0	g
Sodium Lauryl Sulfate	0.1	g
MUG (4-methylumbelliferyl-β-D-glucuronide)	0.05	g

BBL™ Lauryl Sulfate Broth with MUG

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	20.0	g
Lactose	5.0	g
Dipotassium Phosphate	2.75	g
Monopotassium Phosphate	2.75	g
Sodium Chloride	5.0	g
Sodium Lauryl Sulfate	0.1	g
4-Methylumbelliferyl-β-D-glucuronide	0.05	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Lauryl Tryptose Broth with MUG

1. Suspend 35.7 g of the powder in 1 L of purified water and warm slightly to dissolve completely.
2. Dispense into test tubes containing inverted fermentation vials.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Lauryl Sulfate Broth with MUG

1. Dissolve 35.7 g of the powder in 1 L of purified water.
2. Dispense in test tubes, containing inverted Durham tubes, in 10 mL amounts for testing samples of 1 mL or less. For testing 10 mL quantities of samples, dissolve 71.4 g of the powder in 1 L of purified water and distribute in 10 mL amounts. The concentration of the medium should be varied according to the size of the test samples.
3. Autoclave at 121°C, preferably for 12 minutes, but not exceeding 15 minutes. After autoclaving, cool the broth as quickly as possible.
4. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Refrigerated broth generally becomes cloudy or forms precipitates but clears upon warming to room temperature. However, clarity is not important because only gas production is significant.

Procedure

Follow standard methods for the test being performed.[3-5] Observe the medium periodically during the incubation period for the development of fluorescence, using a long-wave UV light source (approximately 366 nm) as well as for characteristic growth and/or gas production.

Expected Results

The presence of *E. coli* is detected by the appearance of fluorescence throughout the tube.

References

1. Feng and Hartman. 1982. Appl. Environ. Microbiol. *43*:1320.
2. Robison. 1984. Appl. Environ. Microbiol. *48*:285.
3. Downes and Ito. 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
5. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Lauryl Tryptose Broth with MUG

AOAC BAM COMPF SMD SMWW

Cat. No.	211740	Dehydrated –100 g
	211744	Dehydrated – 500 g

BBL™ Lauryl Sulfate Broth with MUG

AOAC BAM COMPF SMD SMWW

Cat. No.	298076	Dehydrated –500 g

Lecithin Lactose Agar

Intended Use

Lecithin Lactose Agar is recommended for the isolation and differentiation of histotoxic clostridia from clinical specimens.[1] It is particularly useful in differentiating *Clostridium perfringens*, *C. sordellii*, *C. novyi*, *C. septicum* and *C. histolyticum*.

Summary and Explanation

Culture media containing egg yolks were useful in isolating, cultivating and identifying species of histotoxic clostridia. In 1948, McClung and Toabe reported on the use of egg yolk medium for this purpose.[1] Willis and Hobbs added milk and lactose to the egg yolk in a formulation designed to group clostridia on the basis of production of lecithinase, hydrolysis of casein and lactose fermentation.[2] Neomycin sulfate was included in their formulation in order to make it a selective medium.

Willis, in response to problems in the obtaining and processing of antibiotic-free eggs, developed an egg-free medium in which purified lecithin was substituted for the egg yolk.[3] In order to further refine the growth-promoting and selective properties, Ellner and O'Donnell developed the formulation, designated as Lecithin Lactose Agar, in which a decreased concentration of neomycin was employed and to which sodium azide was added.[4] This medium continues to be an important one for visualizing and characterizing the histotoxic clostridia.

Principles of the Procedure

Lecithin Lactose Agar provides differentiation of clostridia by the demonstration of lecithinase production and lactose fermentation. The medium is rendered selective by the addition of neomycin and sodium azide. Gram-negative and aerobic gram-positive rods are inhibited. Growth of gram-positive cocci is suppressed.

On this medium, the production of a zone of opalescence surrounding colonies indicates lecithinase production. A yellow color surrounding colonies indicates lactose fermentation due to the effect of the lowered pH on the bromcresol purple indicator.

Procedure

As soon as possible after receipt in the laboratory, inoculate the specimen onto a reduced Lecithin Lactose Agar plate and streak for isolation. Since some anaerobes may be inhibited due to the selective nature of the medium, it is advisable to include a nonselective medium such as CDC Anaerobe 5% Sheep Blood Agar.

Media should be reduced prior to inoculation by placing under anaerobic conditions for 6 to 24 hours prior to use.[5] An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak**™ EZ anaerobic systems or an alternative anaerobic system.

Incubate immediately under anaerobic conditions or place in a holding jar flushed with oxygen-free gas(es) until sufficient plates are accumulated (but no longer than 3 hours).[6] Incubation should be at 35 ± 2°C for at least 48 hours. Regardless of anaerobic system used, it is important to include an indicator of anaerobiosis such as a **GasPak** anaerobic indicator.

Expected Results

Plates may be examined after a minimum of 48 hours incubation under anaerobic conditions.

On Lecithin Lactose Agar the production of a zone of opalescence around colonies indicates lecithinase production; a yellow color around colonies indicates lactose fermentation. Typical reactions are listed in the following table.

Legionella Selective Agar

ORGANISM	LECITHINASE PRODUCTION	LACTOSE FERMENTATION
C. perfringens	+	+
C. sordellii	+	–
C. novyi	+	–
C. septicum	–	+
C. histolyticum	–	–

Additional testing may be performed to differentiate the above species.[7,8]

References

1. McClung and Toabe. 1948. J. Bacteriol. *53*:139.
2. Willis and Hobbs. 1959. J. Pathol. Bacteriol. *77*:511.
3. Willis. 1960. J. Pathol. Bacteriol. *80*:379.
4. Ellner and O'Donnell. 1971. Am. J. Clin. Pathol. *56*:197.
5. Allen, Siders and Marler. 1985. Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
6. Martin. 1971. Appl. Microbiol. *22*:1168.
7. Summanen, Baron, Citron, Strong, Wexler and Finegold. 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing Co., Belmont, Calif.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Lecithin Lactose Agar

Cat. No. 221858 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Legionella Agar Base

(See BCYE Agars)

Legionella Selective Agar
Legionella Selective Agar DGVP

Intended Use

These media are used in qualitative procedures for isolation of *Legionella* species from clinical and nonclinical specimens.

Legionella Selective Agar DGVP (dye, glycine, vancomycin, polymixin B) is used for the isolation and differentiation of *Legionella* species from clinical specimens and environmental samples.

Summary and Explanation

Charcoal Yeast Extract (CYE) Agar was developed by Feely et al. in 1979 as a modification of an existing medium for *Legionella* isolation, F-G Agar.[1,2] They replaced the starch in the F-G agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in better recovery of *L. pneumophila*. In 1980, Pasculle reported that CYE Agar could be improved by buffering the medium with ACES Buffer.[3] A year later, Edelstein further increased the sensitivity of the medium by adding alpha-ketoglutarate.[4] BCYE Agar is based on Edelstein's modified formulation.

Legionella Selective Agar was developed by BD Diagnostics for isolation of *Legionella* species from materials containing mixed flora. Consisting of BCYE Agar supplemented with the antimicrobics vancomycin, colistin and anisomycin, it provides improved inhibition of contaminating bacteria without significantly inhibiting *Legionella* species.[5]

Legionella Selective Agar DGVP is a further modification of BCYE Agar incorporating the dyes, bromcresol purple and bromthymol blue.[6] Glycine, vancomycin and polymixin B are included as selective agents.[7]

Principles of the Procedure

These media consist of a base medium (BCYE) supplemented with antibiotics or dyes. Antibiotics improve the recovery of *Legionella* species by inhibiting the growth of contaminating organisms. Dyes facilitate differentiation and identification of *Legionella* species.

The base medium contains yeast extract to supply the nutrients necessary to support bacterial growth. L-cysteine HCL, ferric pyrophosphate and alpha-ketoglutarate are incorporated to satisfy the specific nutritional requirements of *Legionella* species. The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect carbon dioxide and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth of *Legionella* species.

Bromcresol purple and bromthymol blue are included for differentiation of *Legionella* species based on colony morphology and

color.[6] Glycine, vancomycin, and polymyxin B are included to provide moderate inhibition of competing flora and, therefore, enhance the recovery of *Legionella* species.[7]

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar-side up) at 35°C for a minimum of 3 days. Growth is usually visible within 3-days, but may take up to 2 weeks to appear.

Expected Results

On Legionella Selective Agar, *Legionella pneumophila* produces small to large, smooth, colorless to pale blue-gray, slightly mucoid colonies that fluoresce yellow-green when exposed to long-wave UV light. On Legionella Selective Agar DGVP, *L. pneumophila* produces small to large, smooth, pale green colonies which are slightly mucoid.

References

1. Feely, Gibson, Gorman, Langsford, Rasheed, Mackel, and Baine. 1979. J. Clin. Microbiol. *10*:437.
2. Feely, Gorman, Weave, Mackel and Smith. 1978. J. Clin. Microbiol. *8*:320.
3. Pasculle, Feely, Gibson, Cordes, Myerowitz, Patton, Gorman, Carmack, Ezzell and Dowling. 1980. J. Infect. Dis. *191*:727.
4. Edelstein. 1981. J. Clin. Microbiol. *14*:298.
5. Data on file, BD Diagnostic Systems.
6. Vickers, Brown and Garrity. 1981. J. Clin. Microbiol. *13*:380.
7. Wadowski and Yee. 1981. Appl. Environ. Microbiol. *42*:768.

Availability

BBL™ Legionella Selective Agar

Cat. No. 297920 Prepared Plates – Pkg. of 10*

BBL™ Legionella Selective Agar DGVP

Cat. No. 299648 Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*

Leptospira Medium Base EMJH
Leptospira Enrichment EMJH

Intended Use

Leptospira Medium Base EMJH is used with Leptospira Enrichment EMJH in cultivating *Leptospira*.

Summary and Explanation

In 1816, Adolf Weil described the first recognized leptospiral infections in humans.[1] These cases were caused by *Leptospira icterohaemorrhagiae* and the disease was subsequently named Weil's Disease.[1] Leptospirosis is a zoonotic disease, having its reservoir in wild, domestic and peridomestic animals. Infection usually results from direct or indirect exposure to the urine of leptospiruric animals.[2] Indirect exposure through contaminated water and soil accounts for most sporadic cases. Direct exposure occurs in pet owners, veterinarians and persons working with livestock.[3]

The basal medium and enrichment are prepared according to the formulations described by Ellinghausen and McCullough[4] as modified by Johnson and Harris.[5] They modified the formula by replacing rabbit serum medium with polysorbate

User Quality Control

Identity Specifications

Difco™ Leptospira Medium Base EMJH

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	2.3 g of base in 900 mL purified water; soluble upon agitation. Solution is colorless, clear.
Prepared Appearance (with Enrichment):	Very light to light amber, clear.
Reaction (Basal Medium) at 25°C:	pH 7.5 ± 0.2

Difco™ Leptospira Enrichment EMJH

Appearance:	Medium to dark amber, clear to very slightly opalescent.

Cultural Response

Difco™ Leptospira Medium EMJH with Enrichment

Prepare the medium per label directions. Inoculate tubes with undiluted fresh cultures of *Leptospira* and incubate at 30 ± 2°C for up to 7 days.

ORGANISM	ATCC™	INOCULUM	RECOVERY
Leptospira interrogans serotype australis	23605	2-3 Loopfuls	Good
Leptospira interrogans serotype canicola	23470	2-3 Loopfuls	Good
Leptospira kirschneri serotype grippotyphosa	23604	2-3 Loopfuls	Good

Uninoculated Tube

Leptospira interrogans serotype australis ATCC™ 23605

80-albumin. Leptospira Medium EMJH was used in cultivation studies of *Leptospira*.[6]

Leptospira Medium EMJH is recommended for the clinical isolation of *Leptospira* from clinical specimens[7] and environmental samples.[8]

Principles of the Procedure

Leptospira Medium Base EMJH contains ammonium chloride, a nitrogen source, and thiamine, a growth factor. Disodium phosphate and monopotassium phosphate are buffering agents. Sodium chloride provides essential ions.

Leptospira Enrichment EMJH contains albumin, polysorbate 80 and additional growth factors for *Leptospira*.

Formulae

Difco™ Leptospira Medium Base EMJH

Approximate Formula* Per Liter

Disodium Phosphate	1.0	g
Monopotassium Phosphate	0.3	g
Sodium Chloride	1.0	g
Ammonium Chloride	0.25	g
Thiamine	5.0	mg

Difco™ Leptospira Enrichment EMJH

A solution of albumin, polysorbate 80 and additional growth factors for *Leptospira*.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 2.3 g of the powder in 900 mL of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Aseptically add 100 mL Leptospira Enrichment EMJH to the medium at room temperature. Mix thoroughly.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure[7]

Blood and Cerebrospinal Fluid

Freshly drawn blood is preferable; otherwise, use blood taken with SPS, sodium oxalate or heparin.

1. Inoculate four 5 mL tubes of Leptospira Medium EMJH with 1-2 drops of fluid per tube.
2. Incubate in the dark at 28-30°C or at room temperature.

Urine

A total of 12 tubes will be inoculated for each urine specimen.

1. Prepare 1:10 and 1:100 dilutions of urine using Leptospira Medium EMJH to dilute potential inhibitory substances.
2. Inoculate two 5 mL tubes each of Leptospira Medium EMJH with:
   Urine undiluted, 1 drop per tube;
   Urine diluted 1:10, 1 drop per tube;
   Urine diluted 1:100, 1 drop per tube.
3. Duplicate the above inoculations using medium containing 200 µg/mL 5-fluorouracil to inhibit contaminants.
4. Incubate the tubes in the dark at 28-30°C or at room temperature.

Expected Results[7]

1. Examine tubes weekly for signs of growth (turbidity, haze or a ring of growth).
2. Examine tubes microscopically each week. Take a small drop from a few millimeters below the surface, and examine it with dark-field illumination. Use 400 × magnification.
3. Leptospires will be seen as tightly coiled spirochetes about 1 µm wide and 6-20 µm long. Leptospires rotate rapidly on their long axes and usually have hooked ends.
4. If the specimen is positive, subculture about 0.5 mL taken from the area of growth to two tubes of fresh medium.

References

1. Elliott. 1980. J. Am. Med. Tech. *42*:37.
2. Faine (ed.). 1982. Guidelines for the control of leptospirosis. W. H. O. Offset publication no. 67. World Health Organization, Geneva, Switzerland.
3. Weyant, Bragg and Kaufmann. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Ellinghausen and McCullough. 1965. Am. J. Vet. Research *26*:45.
5. Johnson and Harris. 1967. J. Bacteriol. *94*:27.
6. Rule and Alexander. 1986. J. Clin. Microbiol. *23*:500.
7. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.

Availability

Difco™ Leptospira Medium Base EMJH

SMWW

Cat. No. 279410 Dehydrated – 500 g

Difco™ Leptospira Enrichment EMJH

SMWW

Cat. No. 279510 Bottle – 6 x 100 mL*

**Store at 2-8°C.*

L

Letheen Agar • Letheen Broth

Intended Use

Letheen Agar is used to inactivate quarternary ammonium compounds and other preservatives when determining the number of bacteria present in cosmetics and other materials.

Letheen Broth is used for determining the phenol coefficient of cationic surface-active materials.

Summary and Explanation

The value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers was described by Weber and Black[1] in 1948. The addition of lecithin and polysorbate 80 (Tween™* 80) to Tryptone Glucose Extract (TGE) agar resulted in a medium that

**Tween is a trademark of ICI Americas, Inc.*

User Quality Control

Identity Specifications

Difco™ Letheen Agar

Dehydrated Appearance:	Tan, moist appearance, with a few clumps.
Solution:	3.2% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent, may have a slight, fine precipitate (opalescent immediately after autoclaving).
Prepared Appearance:	Light to medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 3.2% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ Letheen Broth

Dehydrated Appearance:	Tan, appears moist, with a tendency to clump.
Solution:	2.57% solution, soluble in purified water upon boiling. Solution is light amber, clear to slightly opalescent (opalescent when hot). May have a very slight precipitate.
Prepared Appearance:	Light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 2.57% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Letheen Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	11229	10^2-10^3	Good
Staphylococcus aureus	6538	10^2-10^3	Good

Difco™ Letheen Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 40- 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	11229	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhi	6539	10^2-10^3	Good
Staphylococcus aureus	6538	10^2-10^3	Good

effectively neutralizes quaternary ammonium compounds in the testing of germicidal activity. Letheen Agar is a modification of TGE agar with the addition of lecithin and polysorbate 80.

Letheen Broth was developed as a subculture medium for the neutralization of quaternary ammonium compounds in disinfectant testing. Quisno, Gibby and Foter[2] found that the addition of lecithin and polysorbate 80 to F.D.A. Broth resulted in a medium that neutralized high concentrations of quaternary ammonium salts. The resulting medium, termed "Letheen" (a combination of Lecithin and Tween), was easy to prepare and clear in appearance, which aided in visual inspection for growth. Letheen Broth is recommended in the *Official Methods of Analysis of AOAC International*[3] for use with disinfectants containing cationic surface active materials.

Letheen Agar and Letheen Broth are specified for use by the American Society for Testing and Materials (ASTM) in the Standard Test Method for Preservatives in Water-Containing Cosmetics.[4]

Principles of the Procedure

Letheen Agar contains beef extract and peptone which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Dextrose is provided as a source of fermentable carbohydrate. Agar is the solidifying agent. Lecithin and polysorbate 80 are added to neutralize surface disinfectants.[2,5,6] Lecithin is added to neutralize quaternary ammonium compounds and polysorbate 80 is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol.[7]

Letheen Broth contains peptone and beef extract which provide the carbon and nitrogen sources necessary for growth. Lecithin and polysorbate 80 are added as surface active disinfectant neutralizing agents.[2,5,6] Sodium chloride is included to maintain osmotic balance.

Formulae

Difco™ Letheen Agar

Approximate Formula* Per Liter

Beef Extract	3.0	g
Pancreatic Digest of Casein	5.0	g
Dextrose	1.0	g
Agar	15.0	g
Polysorbate 80	7.0	g
Lecithin	1.0	g

Difco™ Letheen Broth

Approximate Formula* Per Liter

Beef Extract	5.0	g
Proteose Peptone No. 3	10.0	g
Polysorbate 80	5.0	g
Lecithin	0.7	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Letheen Agar – 32 g;
   Difco™ Letheen Broth – 25.7 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: The dehydrated Letheen Agar has a characteristic "brown sugar" appearance and may seem moist. This does not indicate deterioration.

Procedure

Letheen Agar and Letheen Broth are used in a variety of procedures. Consult appropriate references for further information.[3,4]

Expected Results

Refer to appropriate references and procedures for results.[3,4]

References

1. Weber and Black. 1948. Soap and Sanit. Chem. *24*:134.
2. Quisno, Gibby and Foter. 1946. Am. J. Pharm. *118*:320.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
4. American Society for Testing and Materials. 1991. Standard test method for preservatives in water-containing cosmetics, E 640-78. Annual Book of ASTM Standards. ASTM, Philadelphia, Pa.
5. Erlandson and Lawrence. 1953. Science *118*:274.
6. Brummer. 1976. Appl. Environ. Microbiol. 32:80.
7. Favero (chm.). 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Availability

Difco™ Letheen Agar

Cat. No. 268010 Dehydrated – 500 g*

Difco™ Letheen Broth

AOAC

Cat. No. 268110 Dehydrated – 500 g*

Europe

Cat. No. 257325 Prepared Bottles, 1000 mL – Pkg. of 4

**Store at 2-8°C.*

Letheen Agar, Modified • Letheen Broth, Modified

Intended Use

Letheen Agar, Modified and Letheen Broth, Modified are used for the microbiological testing of cosmetics.

Summary and Explanation

Letheen Agar, Modified and Letheen Broth, Modified are based on Letheen Agar, Modified and Letheen Broth, Modified as described in the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual*.[1] Letheen Agar, Modified and Letheen Broth, Modified are recommended by the FDA for use in the microbiological testing of cosmetics.[1]

User Quality Control

Identity Specifications

Difco™ Letheen Agar, Modified

Dehydrated Appearance:	Tan, moist with a tendency to clump.
Solution:	5.91% solution, soluble in purified water upon boiling. Solution is medium amber, opalescent, may have a slight precipitate. After cooling, slightly opalescent.
Prepared Appearance:	Light-medium amber, slightly opalescent, may have a slight, fine precipitate.
Reaction of 5.91% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Letheen Broth, Modified

Dehydrated Appearance:	Tan, homogeneous, appears moist with a tendency to clump.
Solution:	4.28% solution, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent, may have slight fine precipitate.
Prepared Appearance:	Medium-dark amber, slightly opalescent, may have a slight fine precipitate.
Reaction of 4.28% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Letheen Agar, Modified or Letheen Broth, Modified

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AGAR	RECOVERY BROTH
Staphylococcus aureus	6538	25-100	Good	Good
Salmonella enterica subsp. *enterica* serotype Typhi	6539	25-100	N/A	Good

Principles of the Procedure

Beef extract, included in the Letheen Agar and Letheen Broth bases, and peptone provide carbon and nitrogen sources required for good growth of a wide variety of bacteria and fungi. The peptone level was increased in the modified Letheen Agar and Broth formulas to provide for better growth. Vitamins and cofactors, required for growth as well as additional sources of nitrogen and carbon, are provided by yeast extract. Sodium chloride provides a suitable osmotic environment. In Letheen Broth, Modified sodium chloride is provided by the Letheen Broth component. Both media also contain polysorbate 80, lecithin and sodium bisulfite to partially neutralize the preservative systems commonly found in cosmetics. Additional agar is included in Letheen Agar, Modified as the solidifying agent.

Formulae

Difco™ Letheen Agar, Modified

Approximate Formula* Per Liter		
Letheen Agar	32.0	g
Tryptone	5.0	g
Proteose Peptone No. 3	10.0	g
Yeast Extract	2.0	g
Sodium Chloride	5.0	g
Sodium Bisulfite	0.1	g
Agar	5.0	g

Difco™ Letheen Broth, Modified

Approximate Formula* Per Liter		
Letheen Broth	25.7	g
Tryptone	5.0	g
Proteose Peptone No. 3	10.0	g
Yeast Extract	2.0	g
Sodium Bisulfite	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Letheen Agar, Modified - 59.1 g;
   Difco™ Letheen Broth, Modified - 42.8 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure[1]

1. Prepare and dilute samples in Letheen Broth, Modified in accordance with established guidelines.
2. Using the spread plate technique, inoculate in duplicate 0.1 mL of the diluted samples onto Letheen Agar, Modified, Potato Dextrose Agar (or Malt Extract Agar) containing chlortetracycline, Baird-Parker Agar (or Vogel-Johnson Agar, optional), Anaerobic Agar, and a second set of Letheen Agar, Modified plates.
3. Incubate one set of Letheen Agar, Modified plates at 30 ± 2°C for 48 hours and the other set at 35 ± 2°C under anaerobic conditions for 2-4 days. Incubate the Potato Dextrose Agar (or Malt Extract Agar) plates at 30 ± 2°C for 7 days and the Baird-Parker Agar (or Vogel-Johnson Agar) plates, if inoculated, at 35 ± 2°C for 48 hours.
4. Incubate the diluted samples from step 1 at 35 ± 2°C for 7 days. Subculture enriched samples onto Letheen Agar, Modified only if there is no growth on the primary Letheen Agar, Modified plates.

Expected Results

Examine plates for evidence of growth and characteristic colonial morphology. Determine colony counts and subculture each colony type onto Letheen Agar, Modified and MacConkey Agar (also Baird- Parker or Vogel-Johnson Agar, if used in step 2).

Determine Gram reaction, cell morphology and catalase reactions. Identify bacterial isolates in accordance with established procedures.[1]

Reference

1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Letheen Agar, Modified

BAM

Cat. No. 263110 Dehydrated – 500 g*

Europe

Cat. No. 257452 Sterile Pack **RODAC**™ Plates – Ctn. of 100*
257451 Prepared Plates (sterile) – Ctn. of 100*

Difco™ Letheen Broth, Modified

BAM

Cat. No. 263010 Dehydrated – 500 g*

Europe

Cat. No. 257327 Prepared Bottles, 500 mL – Pkg. of 4

**Store at 2-8°C.*

Levine EMB Agar

(See Eosin Methylene Blue Agar, Levine)

Lim Broth

Intended Use

Lim Broth is used for the selective enrichment of group B streptococci (*Streptococcus agalactiae*), especially from genital specimens.

Summary and Explanation

Since its emergence in the 1970s, neonatal group B streptococcal disease has become the major infectious cause of illness and death among newborns. Prior to 1994, an estimated 7600 episodes of invasive group B streptococcal disease, primarily sepsis and meningitis, occurred in newborns each year in the United States, with approximately 80% of those episodes representing early-onset disease occurring within the first week of life.[1] The disease is spread to newborns through vertical transmission from a mother who carries B streptococci in her anorectum or genital tract. Lim and colleagues combined the use of an enriched, selective broth medium and slide coagglutination test to rapidly screen such maternity patients.[2-5]

The Centers for Disease Control and Prevention (CDC) has published guidelines for screening and use of intrapartum chemoprophylaxis for prevention of neonatal group B streptococcal disease.[6] The use of Todd Hewitt Broth with colistin and nalidixic acid is a medium recommended to maximize the likelihood of recovering group B streptococci upon plating on sheep blood agar.[1] Lim Broth is prepared from Todd Hewitt Broth by the addition of colistin and nalidixic acid, at the recommended concentrations, plus yeast extract for enhanced growth of group B streptococci.[2]

Group B streptococci have also been found in cases of sepsis in nonparturient women and men, and in joint infections, osteomyelitis, urinary tract infections and wound infections. They are associated with endocarditis, pneumonia and pyelonephritis in immunosuppressed patients.[7]

Principles of the Procedure

Todd Hewitt Broth base is a general-purpose medium primarily used for the cultivation of β-hemolytic streptococci, especially for serologic studies.[8]

The peptones, dextrose and salts provide an excellent nutritional base for the growth of streptococci. The added yeast extract is a rich source of B-complex vitamins. Dextrose stimulates hemolysin production. Disodium phosphate and sodium carbonate provide buffering action to counteract the acidity

produced during the fermentation of the carbohydrate, thereby protecting the hemolysin from inactivation by the acid.

Nalidixic acid and colistin suppress growth of gram-negative bacteria.

Procedure

Inoculate tubes and incubate with loosened caps at 35 ± 2°C in an aerobic atmosphere with or without added carbon dioxide. If desired, perform a slide coagglutination test for group B streptococci after 5 hours of incubation.[4]

If turbidity is observed after 18-24 hours, subculture from the broth culture to a sheep blood agar plate; otherwise, incubate an additional 24 hours before discarding.[1]

Expected Results

Growth in broth medium is indicated by the presence of turbidity compared to an uninoculated control.

Subculture to a **Trypticase**™ Soy Agar with 5% Sheep Blood (TSA II) plate and incubate for 18-24 hours, or up to 48 hours if necessary. Identify organisms suggestive of group B streptococci (β- or non-hemolytic, gram-positive and catalase-negative). Specific identification may be performed; e.g., using streptococcal grouping sera, the CAMP test or other procedures.

Jones et al. reported detection of group B streptococci by slide coagglutination after 5 hours of incubation when the concentration of organisms in the culture was 10^7 per mL or greater.[4]

References

1. Federal Register. 1994. Prevention of group B streptococcal disease: a public health perspective. Fed. Regist. *59*:64764.
2. Jones, Friedl, Kanarek, Williams and Lim. 1983. J. Clin. Microbiol. *18*:558.
3. Lim, Kanarek and Peterson. 1982. Curr. Microbiol. *7*:99.
4. Jones, Kanarek and Lim. 1984. J. Clin. Microbiol. *20*:438.
5. Jones, Kanarek, Angel and Lim. 1983. J. Clin. Microbiol. *18*:526.
6. Centers for Disease Control and Prevention. 2002. Morbid. Mortal. Weekly Rep. *51* (No. RR-11):1.
7. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby Inc., St. Louis, Mo.
8. Todd and Hewitt. 1932. J. Pathol. Bacteriol. *35*:973.

Availability

BBL™ Lim Broth

BS12 CMPH2 MCM9

Cat. No.	292209	Prepared Tubes (K Tubes), 5 mL – Pkg of 10*
	296266	Prepared Tubes (K Tubes), 5 mL – Ctn. of 100*

**Store at 2-8°C.*

Listeria Enrichment Broth • Listeria Enrichment Broth, Modified • Buffered Listeria Enrichment Broth Base

Intended Use

Listeria Enrichment Broth is used to selectively enrich *Listeria* from foods.

Listeria Enrichment Broth, Modified is used for selectively enriching *Listeria* from raw and pasteurized milk.

Buffered Listeria Enrichment Broth Base is used as an enrichment broth for the cultivation of *Listeria* spp. from food according to the U.S Food and Drug Administration (FDA).[1]

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[2] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[3] The first reported foodborne outbreak of listeriosis was in 1985.[4] Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[5]

Implicated vehicles of transmission include turkey frankfurters,[6] coleslaw, pasteurized milk, Mexican-style cheese, paté, and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[7]

Listeria species grow over a pH range of 4.4-9.6, and survive in food products with pH levels outside these parameters.[8] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[9] Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Listeria Enrichment Broth is based on the formula developed by Lovett et al.[10] in which Tryptic Soy Broth is supplemented with yeast extract for optimum growth of *Listeria*. Listeria Enrichment Broth, Modified is a modification of Listeria Enrichment Broth in which the concentration of one of the selective agents, acriflavine, has been reduced from 15 mg to 10 mg per liter. This modification reflects the lower concentration once specified by the IDF for isolation of *L. monocytogenes* from milk and milk products (IDF Standard No. 143A).[11] More recently, the IDF standard has been replaced by ISO Standard 11290 which utilizes Half Fraser Broth for enrichment.[12]

Buffered Listeria Enrichment Broth Base is a modification of Listeria Enrichment Broth with added buffering strength. The addition of selective agents is delayed until after four hours of enrichment with this formula.[1]

User Quality Control

Identity Specifications

Difco™ Listeria Enrichment Broth or Listeria Enrichment Broth, Modified

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.61% solution, soluble in purified water upon boiling. Solution is light to medium yellowish-amber with a faint green ring at the surface, clear to very slightly opalescent.
Prepared Appearance:	Light yellowish-amber, clear to slightly opalescent.
Reaction of 3.61% Solution at 25°C:	pH 7.3 ± 0.2

Difco™ Buffered Listeria Enrichment Broth Base

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.8% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 4.8% Solution at 25°C:	pH 7.3 ± 0.1

Cultural Response

Difco™ Listeria Enrichment Broth

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	2×10^3-10^4	Inhibition at 18-24 hours; none to poor at 40-48 hours
Escherichia coli	25922	2×10^3-10^4	Inhibition
Listeria monocytogenes	19114	10^2-10^3	Good
Saccharomyces cerevisiae	9080	2×10^3-10^4	Inhibition

Difco™ Listeria Enrichment Broth, Modified

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	2×10^3-10^4	Partial inhibition
Escherichia coli	25922	2×10^3-10^4	Marked to complete inhibition
Listeria monocytogenes	19114	10^2-10^3	Good
Listeria monocytogenes	19115	10^2-10^3	Good
Saccharomyces cerevisiae	9080	2×10^3-10^4	Marked to complete inhibition

Difco™ Buffered Listeria Enrichment Broth Base

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 4 hours. After 4 hours of incubation, aseptically add filter-sterilized selective agents according to the label directions. Reincubate at 30 ± 2°C and observe for growth after 48 hours of total incubation.

ORGANISM	ATCC™	INOCULUM CFU	GROWTH
Escherichia coli	25922	10^3	Inhibition
Listeria monocytogenes	19114	30-300	Good
Listeria monocytogenes	19115	30-300	Good
Saccharomyces cerevisiae	9080	10^3	Inhibition

Principles of the Procedure

Peptones and yeast extract provide nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Sodium chloride maintains the osmotic balance of the medium. Phosphates provide buffering capacity. Sodium pyruvate aids in resuscitation of stressed organisms. Nalidixic acid inhibits growth of gram-negative organisms. Acriflavine HCl suppresses the growth of gram-positive bacteria. Cycloheximide is incorporated to inhibit saprophytic fungi.

Formulae

Difco™ Listeria Enrichment Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	17.0	g
Soytone	3.0	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Yeast Extract	6.0	g
Cycloheximide	0.05	g
Acriflavine HCl	15.0	mg
Nalidixic Acid	0.04	g

Difco™ Listeria Enrichment Broth, Modified

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	17.0	g
Soytone	3.0	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Yeast Extract	6.0	g
Cycloheximide	0.05	g
Acriflavine HCl	10.0	mg
Nalidixic Acid	0.04	g

Difco™ Buffered Listeria Enrichment Broth Base

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	17.0	g
Soytone	3.0	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Disodium Phosphate	9.6	g
Monopotassium Phosphate	1.35	g
Yeast Extract	6.0	g
Sodium Pyruvate	1.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Listeria Enrichment Broth – 36.1 g;
   Difco™ Listeria Enrichment Broth, Modified – 36.1 g;
   Difco™ Buffered Listeria Enrichment Broth Base – 48 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.

4. For Buffered Listeria Enrichment Broth Base, prepare filter-sterilized solutions of the following agents and add to the base as directed under "Procedure":
   1% (w/v) cycloheximide in 40% (v/v) solution of ethanol in water;
   0.5% (w/v) acriflavine HCl in purified water;
   0.5% (w/v) nalidixic acid in purified water.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Listeria Enrichment Broth

For food samples, use Listeria Enrichment Broth in recommended laboratory procedures for isolating *Listeria*.

Listeria Enrichment Broth, Modified

For dairy samples, a selective enrichment method is as follows or consult appropriate references.[1,8,14]

1. Add 25 mL of liquid or 25 g of solid test material to 225 mL Listeria Enrichment Broth, Modified and mix or blend thoroughly.
2. Incubate for 48 hours at 30°C.
3. At 48 hours, streak the Listeria Enrichment Broth, Modified culture onto plates of Oxford Medium or PALCAM Medium.
4. Incubate the agar plates at 37°C for 48 ± 2 hours.

Buffered Listeria Enrichment Broth Base

For food samples, the FDA[1] selective enrichment method is as follows:

1. Add 25 mL liquid or 25 g solid test material to 225 mL Buffered Listeria Enrichment Broth Base without selective agents and mix or blend thoroughly.
2. Incubate for 4 hours at 30°C.
3. Add 0.455 mL acriflavine HCl solution, 1.8 mL nalidixic acid solution and 1.15 mL cycloheximide solution to 225 mL Buffered Listeria Enrichment Broth Base and continue incubating another 44 hours, for a total of 48 hours, at 30°C.
4. At 24 and 48 hours incubation, streak incubated broth onto both Oxford Medium and LPM Agar (or PALCAM Agar) plates. Incubate Oxford Medium and PALCAM plates at 35°C for 24-48 hours, and LPM plates at 30°C for 24-48 hours.

Expected Results

1. Examine agar plates for typical *Listeria* colonies.
2. Consult appropriate references for selection of biochemical and/or serological tests for confirmation of *Listeria* spp.[1,8,13,14]

Limitation of the Procedure

Listeria Enrichment Broth, Modified is a partially selective medium. Growth of some contaminating strains will be markedly but not totally inhibited.

References

1. Hitchens. 2001. *In* FDA bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
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3. Monk, Clavero, Beuchat, Doyle and Brackett. 1994. J. Food Prot. *57*:969.
4. Wehr. 1987. J. Assoc. Off. Anal. Chem. *70*:769.
5. Bremer and Osborne. 1995. J. Food Prot. *58*:604.
6. Grau and Vanderlinde. 1992. J. Food Prot. *55*:4.
7. Patel, Hwang, Beuchat, Doyle and Brackett. 1995. J. Food Prot. *58*:244.
8. Ryser and Donnelly. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
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11. International Dairy Federation. 1995. Milk and milk products—detection of *Listeria monocytogenes*. International IDF Standard No. 143A. International Dairy Federation, Brussels, Belgium.
12. International Organization for Standardization. 1996. ISO standard 11290-1:1996: Microbiology of food and animal feeding stuffs—horizontal method for the detection and enumeration of *Listeria monocytogenes*, Part 1: Detection method, ISO, Geneva, Switzerland.
13. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
14. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Listeria Enrichment Broth

Cat. No.	222220	Dehydrated – 500 g
	222210	Dehydrated – 10 kg

Difco™ Listeria Enrichment Broth, Modified

Cat. No.	220530	Dehydrated – 500 g
	245152	Dehydrated – 2 kg
	220520	Dehydrated – 10 kg

Difco™ Buffered Listeria Enrichment Broth Base

BAM COMPF SMD

Cat. No.	290720	Dehydrated – 500 g
	214919	Dehydrated – 2 kg

L

Litmus Milk

Intended Use

Litmus Milk is used for the maintenance of lactic acid bacteria and as a differential medium for determining the action of bacteria on milk.

Summary and Explanation

Litmus Milk has been used for many years for determining the metabolic activities of microorganisms in milk as an aid to the identification of bacterial species. It is especially useful in species differentiation within the genus *Clostridium*.

This medium is also of value in the maintenance and propagation of lactic bacteria.

User Quality Control

Identity Specifications

BBL™ Litmus Milk

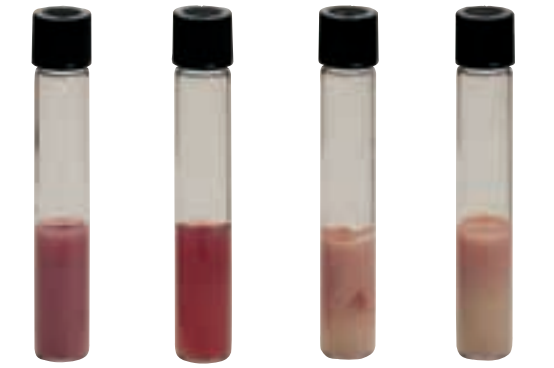
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	10.0% solution, soluble in purified water. Solution is medium, purple gray, opaque.
Prepared Appearance:	Medium, purple gray, opaque.
Reaction of 10.0% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Litmus Milk

Prepare the medium per label directions. Inoculate with fresh cultures diluted 1:10 and incubate at 35 ± 2°C for 7 days.

ORGANISM	ATCC™	RESULT
Clostridium perfringens	13124	Stormy fermentation (gas), clot or curd, reduction (white)
Lactobacillus acidophilus	4356	Acid (pink), clot or curd



Uninoculated Tube | *Bacillus subtilis* ATCC™ 6633 | *Clostridium perfringens* ATCC™ 12924 | *Lactobacillus rhamnosus* ATCC™ 7469

Principles of the Procedure

Skim milk is the substrate that particular species of bacteria attack in different ways to produce various metabolic products. Azolitmin serves as a pH indicator with a color range of pink (below pH 4.5) to purple (in middle of pH range) to blue (above pH 8.3) and also functions as an Eh (oxidation-reduction) indicator.[1]

The action of bacteria on milk can be categorized as follows:

1. No change (no carbohydrate fermentation and no change of litmus indicator).
2. Fermentation of lactose and/or dextrose in the milk with production of acid (pink color), including stormy fermentation (strong evolution of gas) by certain strains of *Clostridium*.
3. Action of proteolytic enzymes on lactalbumin with production of ammonia or basic amines resulting in an alkaline reaction (blue color).
4. Coagulation of casein as evidenced by the formation of a curd or clot. If the casein is converted to paracasein by the enzyme rennin, a clear, watery liquid called "whey" is produced at the top of a thoroughly coagulated tube.[1]
5. Peptonization due to digestion of the milk protein as evidenced by a clearing of the medium and dissolution of the clot.
6. Reduction of the litmus in the depths of the tube due to the action of reductase enzymes with the resultant removal of oxygen to form the decolorized leucolitmus compound.

Formula

BBL™ Litmus Milk

Approximate Formula* Per Liter

Skim Milk	100.0	g
Azolitmin	0.5	g
Sodium Sulfite	0.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 100 g of the powder in 1 L of purified water, preferably preheated to 50°C. Mix thoroughly.
2. Autoclave at 115°C for 20 minutes or by fractional steam sterilization for 30 minutes on three successive days. Avoid overheating and the consequent caramelization which occurs.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate tubes of Litmus Milk with 18- to 24-hour pure cultures. For the study of anaerobic organisms, sterile mineral oil can be layered over the medium following inoculation. Incubate tubes at 35 ± 2°C for up to 14 days and record reactions at various intervals during the incubation process.

Expected Results

Consult an appropriate reference for the expected reactions for specific microbial species.[2]

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Sneath and Holt (ed.). 1986. Bergey's Manual™ of determinative bacteriology, vol. 2. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Litmus Milk

Cat. No.	211343	Dehydrated – 500 g
	221657	Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*

Liver Infusion Agar • Liver Infusion Broth

Intended Use

Liver Infusion Agar is used for cultivating *Brucella* and other pathogenic organisms.

Liver Infusion Broth is used for cultivating a variety of organisms, particularly *Brucella* and anaerobes.

Summary and Explanation

Brucellosis is a zoonotic disease with a domestic animal reservoir. Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[1]

Most strains of *Brucella* will grow on chocolate or blood agar. However, special media such as liver infusion, tryptose, tryptone or brucella agar are preferred.[2] The nutritive factors of Liver Infusion media permit luxuriant growth of *Brucella* and other fastidious pathogens.

For isolating *Brucella* strains from contaminated milk, crystal violet (gentian violet) can be added to Liver Infusion Agar to suppress gram-positive organisms.[3] Five percent (5%) heated horse or rabbit serum enhances growth of *Brucella*.[4]

User Quality Control

Identity Specifications

Difco™ Liver Infusion Agar

Dehydrated Appearance:	Dark beige to light tan, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in purified water upon boiling. Solution is medium to dark amber, slightly opalescent to opalescent.
Prepared Appearance:	Medium to dark amber, slightly opalescent.
Reaction of 5.5% Solution at 25°C:	pH 6.9 ± 0.2

Difco™ Liver Infusion Broth

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water. Solution is medium to dark amber, clear to very slightly opalescent with a few particles.
Prepared Appearance:	Medium to dark amber, clear to very slightly opalescent with a few particles.
Reaction of 3.5% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Liver Infusion Agar or Liver Infusion Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours, or up to 72 hours if necessary. Incubate *Clostridium* under anaerobic conditions. Incubate *Brucella* spp. and *S. pneumoniae* with 3-5% CO_2.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella abortus	11192*	30-300	Good
Brucella melitensis	4309*	30-300	Good
Brucella suis	4314*	30-300	Good
Clostridium sporogenes	11437	30-300	Good
Streptococcus pneumoniae	6305	30-300	Good

**Minimally, one strain of* Brucella *should be used for performance testing. These ATCC strains should be used if available.*

Liver Infusion Agar at approximately half strength may be used to prepare Endamoeba medium for cultivating *Endamoeba histolytica*.[5]

Liver Infusion Broth maintains a degree of anaerobiosis well suited to support growth of anaerobic microorganisms, especially *Clostridium* species.

Principles of the Procedure

Peptones and infusions provide the nitrogen, amino acids, vitamins and carbon sources in Liver Infusion media. Sodium chloride maintains the osmotic balance. Agar is the solidifying agent.

Formulae

Difco™ Liver Infusion Agar

Approximate Formula* Per Liter		
Beef Liver, Infusion from 500 g	20.0	g
Proteose Peptone	10.0	g
Sodium Chloride	5.0	g
Agar	20.0	g

Difco™ Liver Infusion Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions[6]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ Liver Infusion Agar – 55 g;
   Difco™ Liver Infusion Broth – 35 g.
   Mix thoroughly.
2. Heat the Liver Infusion Agar with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion of the isolation and identification of *Brucella,* anaerobic microorganisms and other fastidious pathogens, refer to the procedures described in *Bailey & Scott's Diagnostic Microbiology*,[4] *Clinical Microbiology Procedures Handbook*[7] and *Manual of Clinical Microbiology*.[8]

Expected Results
Refer to appropriate references and procedures for results.

References
1. Shapiro and Wong. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Carter. 1979. Diagnostic procedures in veterinary bacteriology and mycology, 3rd ed. Charles C. Thomas, Springfield, Ill.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
5. Cleveland and Sanders. 1930. Arch. Protietenkd. *70*:223.
6. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
7. Isenberg and Garcia. (ed.). 2004. (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability
Difco™ Liver Infusion Agar
Cat. No. 252100 Dehydrated – 500 g

Difco™ Liver Infusion Broth
Cat. No. 226920 Dehydrated – 500 g

Liver Veal Agar

Intended Use
Liver Veal Agar is used for cultivating anaerobic bacteria.

Summary and Explanation
Spray[1] described a procedure using the anaerobic culture dish for the cultivation of these organisms. Liver Veal Agar is identical to the medium described by Spray.[2] Liver Veal Agar provides a rich supply of nutrients for anaerobic and fastidious aerobic pathogens. The medium supports excellent growth of sporulating anaerobes and can be used for deep tube cultures.

User Quality Control

Identity Specifications
Difco™ Liver Veal Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	9.7% solution, soluble in purified water upon boiling. Solution is medium to dark amber, opalescent, may have a slight precipitate.
Prepared Appearance:	Medium to dark amber, opalescent, may have a slight precipitate.
Reaction of 9.7% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response
Difco™ Liver Veal Agar
Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours under appropriate atmospheric conditions. Incubate clostridia anaerobically, *Neisseria* under increased CO_2 and streptococci aerobically.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Clostridium botulinum	3502*	10^2-10^3	Good
Clostridium tetani	10779*	10^2-10^3	Good
Neisseria meningitidis	13090	10^2-10^3	Good
Streptococcus pneumoniae	6305	10^2-10^3	Good

**Minimally, one strain of these clostridia should be used for performance testing. These ATCC strains should be used if available.*

Liver Veal Agar is specified in the FDA *Bacteriological Analytical Manual* (BAM)[3] and *Compendium of Methods for the Microbiological Examination of Foods*.[4] Liver Veal Agar can be supplemented with 50% egg yolk for the cultivation of anaerobic organisms.[3-5]

Principles of the Procedure
Infusions, peptones, gelatin and isoelectric casein provide the rich nitrogen, amino acids and vitamin content of the medium. Soluble starch is added to enhance the growth of anaerobes and dextrose is a carbon source. Sodium chloride maintains osmotic balance and agar is the solidifying agent.

Precautions[6]
1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing *C. botulinum* or *C. tetani* or their toxins.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of *C. botulinum* and for activities with a high potential for aerosol or droplet production, and those involving production quantities of toxin.

Formula
Difco™ Liver Veal Agar

Approximate Formula* Per Liter		
Liver, Infusion from 50 g	9.0	g
Veal, Infusion from 500 g	6.4	g
Proteose Peptone	20.0	g
Gelatin	20.0	g
Soluble Starch	10.0	g
Isoelectric Casein	2.0	g
Dextrose	5.0	g
Neopeptone	1.3	g
Tryptone	1.3	g
Sodium Chloride	5.0	g
Sodium Nitrate	2.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 97 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion of the isolation and identification of anaerobic bacteria and other fastidious aerobic pathogens, refer to the procedures described in *Clinical Microbiology Procedures Handbook*[7] and *Manual of Clinical Microbiology*.[8]

Expected Results

Refer to appropriate references and procedures for results.

References

1. Spray. 1930. J. Lab. Clin. Med. *16*:203.
2. Spray. 1936. J. Bacteriol. *32*:135.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. Atlas. 1993. Handbook of microbiological media. CRC Press, Boca Raton, Fla.
6. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
7. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Liver Veal Agar

BAM CCAM COMPF

Cat. No. 259100 Dehydrated – 500 g

Lowenstein Media
Lowenstein Medium Base • Lowenstein-Jensen Medium • Lowenstein-Jensen Medium, Gruft Lowenstein-Jensen Medium with Iron Lowenstein-Jensen Medium with Pyruvic Acid Lowenstein-Jensen Medium with 5% Sodium Chloride

Intended Use

Lowenstein Medium and Lowenstein-Jensen (LJ) Medium are used for the isolation and cultivation of mycobacteria and as bases for selective, differential and enriched media for mycobacteria.

LJ Medium, tubed as deeps, is used for the semi-quantitative catalase test.

LJ Medium, Gruft, is a selective medium used for the isolation and cultivation of mycobacteria.

LJ Medium with Iron is used to determine iron uptake for differentiation and identification of mycobacteria.

LJ Medium with Pyruvic Acid is an enrichment medium used for enhanced growth of mycobacteria.

LJ Medium with 5% sodium chloride is used to characterize certain strains of mycobacteria.

Summary and Explanation

LJ Medium is an inspissated, egg-based medium developed from Jensen's modification of Lowenstein's formula.[1,2]

Gruft modified LJ Medium by adding penicillin and nalidixic acid for selective isolation of mycobacteria.[3] Gruft also found that the addition of ribonucleic acid (RNA) increased the percentage of tubercle bacilli recovered from clinical specimens compared to recovery on the standard LJ Medium.[4]

Wayne and Doubek differentiated rapidly-growing from slow-growing mycobacteria based on iron intake.[5] The rapid-growing mycobacteria take up iron in the medium, producing rusty-brown colonies and a tan discoloration in the medium.[6] *M. chelonae* and slow-growing species do not take up the iron.[7]

Hughes [8] and Dixon and Cuthbert [9] reported that the addition of pyruvic acid to egg-based media resulted in improved recovery of tubercle bacilli compared to recovery on egg-based media supplemented only with glycerol. Dixon and Cuthbert recommended using pyruvic acid-egg medium in addition to media supplemented with glycerol for optimum recovery of tubercle bacilli from clinical specimens.[9]

Additionally, the medium is available with the addition of 5% sodium chloride. Most rapid growers, the slowly growing *M. triviale* and some strains of *M. flavescens* grow on NaCl-containing media. The inability of *M. chelonae* subsp. *chelonae* to grow helps differentiate it from other members of the *M. fortuitum* complex (e.g., *M. chelonae* subsp. *abscessus*).[6,10]

In the semi-quantitative catalase test, mycobacteria can be differentiated into groups, based upon catalase activity. [6,11,12]

User Quality Control

Identity Specifications

Difco™ Lowenstein Medium Base

Dehydrated Appearance:	Medium to dark green-blue, free flowing, homogeneous.
Solution:	37.4 g/600 mL solution containing 12 mL of glycerol, soluble in purified water upon boiling. Solution is opalescent, viscous, dark blue-green.
Prepared Appearance:	Opalescent, viscous, dark blue green.

Cultural Response

Difco™ Lowenstein Medium Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for up to 21 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-2×10^3	Partial inhibition
Mycobacterium tuberculosis H37Ra	25177	10^2-3×10^2	Good
Mycobacterium tuberculosis	27294	10^2-3×10^2	Good
Mycobacterium kansasii Group I	12478	10^2-3×10^2	Good
Mycobacterium scrofulaceum Group II	19981	10^2-3×10^2	Good
Mycobacterium intracellulare Group III	13950	10^2-3×10^2	Good
Mycobacterium fortuitum Group IV	6841	10^2-3×10^2	Good

Uninoculated Tube

Mycobacterium fortuitum ATCC™ 6841

Principles of the Procedure

Lowenstein Medium Base is a relatively simple formulation that requires supplementation in order to support the growth of mycobacteria. Glycerol and egg mixture are added prior to the inspissation process. These substances provide fatty acids and protein required for the metabolism of mycobacteria. The coagulation of the egg albumin during sterilization provides a solid medium for inoculation purposes. Malachite green selectively inhibits contaminants.

Low-level concentrations of penicillin (50.0 units/mL) and nalidixic acid (35.0 mg/mL) are included in the LJ Medium, Gruft, to inhibit gram-positive as well as some gram-negative bacterial contaminants. The addition of RNA (0.05 mg/mL) enhances the recovery of tubercle bacilli.

In the iron uptake test, most rapid growers take up the iron salt in the medium (ferric ammonium citrate, 25 mg/mL), producing rusty brown colonies and a tan discoloration in the surrounding medium. Slow-growing species and most strains of *M. chelonae* do not take up the iron in the medium.[6,7]

Pyruvic acid (2.5 mg/mL) enhances the growth of tubercle bacilli.

The ability to tolerate 5% sodium chloride is a characteristic of certain strains of mycobacteria (e.g., *M. fortuitum* and *M. chelonae* subsp. *abscessus*).[10]

Catalase is an intracellular, soluble enzyme capable of splitting hydrogen peroxide into water and oxygen. The oxygen bubbles into the reaction mixture creating a column of bubbles. With a column height breakpoint of 45 mm, the mycobacteria can be divided into groups: those producing less than 45 mm (*M. tuberculosis, M. marinum, M. avium* complex and *M. gastri*); and those producing more than 45 mm *(M. kansasii, M. simiae*, most scotochromogens, the nonphotochromogenic saprophytes and the rapid growers).[6]

Formula

Difco™ Lowenstein Medium Base

Approximate Formula* Per 600 mL		
Asparagine	3.6	g
Monopotassium Phosphate	2.4	g
Magnesium Sulfate	0.24	g
Magnesium Citrate	0.6	g
Potato Flour	30.0	g
Malachite Green	0.4	g

**Adjusted and/or supplemented as required to meet performance criteria*

Precaution[13]

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Directions for Preparation from Dehydrated Product

1. Suspend 37.4 g of the powder in 600 mL of purified water containing 12 mL of glycerol. Do not add glycerol if bovine tubercle bacilli or other glycerophobic organisms are to be cultivated. Mix thoroughly.
2. Heat with frequent agitation just until the medium boils.
3. Autoclave at 121°C for 15 minutes. Cool to approximately 50°C.
4. Meanwhile, prepare 1,000 mL of whole eggs collected aseptically and mixed thoroughly, without introducing air bubbles.
5. Admix base and egg gently until mixture is uniform and without bubbles.

6. Distribute in suitable sterile containers such as screw-capped tubes.
7. Arrange tubes in slanted position, then coagulate and inspissate at 85°C for 45 minutes.
8. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[6] N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. These reagents are provided in the **BBL™ MycoPrep™** Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[6,7,12,14,15]

Following inoculation, keep test containers shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C.

Slanted and bottled media should be incubated in a horizontal plane until the inoculum is absorbed. Tubes and bottles should have screw caps loose for the first 3 weeks to permit the circulation of carbon dioxide for the initiation of growth. Thereafter, to prevent dehydration, tighten caps; loosened briefly once a week. Stand tubes upright if space is a problem.

NOTE: Cultures from skin lesions suspected to be *M. marinum* or *M. ulcerans* should be incubated at 25-33°C for primary isolation; cultures suspected to contain *M. avium* or *M. xenopi* exhibit optimum growth at 40-42°C.[6] Incubate a duplicate culture at 35-37°C.

For LJ Medium with Iron, specimens must first be isolated in pure culture on an appropriate solid medium. Inoculate LJ Medium with Iron with one drop of a barely turbid suspension of the culture to be tested.

For the semi-quantitative catalase test, 1 mL of a 1:1 mixture of 10% polysorbate 80 and 30% hydrogen peroxide is added to each inoculated tube after 2 weeks of incubation. The height of the column of bubbles is recorded after 5 minutes as <45 mm or >45 mm.[6,7]

Expected Results

Cultures should be read within 5-7 days after inoculation and once a week thereafter for up to 8 weeks.

Record Observations:

1. Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7 days; slow growers require more than 7 days for mature colony forms.
2. Pigment production
   White, cream to buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.

Bottles may be examined by inverting the bottles on the stage of a dissecting microscope. Read at 10-60× with transmitted light. Scan rapidly at 10-20× for the presence of colonies. Higher magnification (30-60×) is helpful in observing colony morphology; i.e., serpentine cord-like colonies.

Examine LJ Medium with Iron for rusty-brown colonies with a tan discoloration in the surrounding medium, indicating uptake of the iron.

The presence or absence of growth in the tube of medium containing 5% NaCl aids in the differentiation of mycobacterial isolates. The salt tolerance test is positive when numerous colonies appear on the control medium and more than 50 colonies grow on the medium containing 5% NaCl.[6,15] Colonies on the control medium, but no visible growth on the test medium after a total of 4 weeks of incubation constitutes a negative test.[6,12,15]

In the semi-quantitate catalase test, mycobacteria fall into two groups with *M. tuberculosis* falling into the group producing a column of bubbles less than 45 mm in height.[6]

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

1. Lowenstein. 1931. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig.*120*:127.
2. Jensen. 1932. Zentralb. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. *125*:222.
3. Gruft. 1971. Health Lab. Sci. *8*:79.
4. Gruft. 1963. Am. Rev. Respir. Dis. *88*:412.
5. Wayne and Doubek. 1968. Appl. Microbiol. *16*:925.
6. Kent and Kubica. 1985. Public health mycobacteriology: a guide to the level III laboratory. USDHHS. Centers for Disease Control, Atlanta, Ga.
7. Metchock, Nolte and Wallace. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
8. Hughes. 1966. J. Clin. Pathol. *19*:73.
9. Dixon and Cuthbert. 1967. Am. Rev. Respir. Dis. *96*:119.
10. Silcox, Good and Floyd. 1981. J. Clin. Micobiol. *14*:686.
11. Wayne. 1962. Am. Rev. Respir. Dis. *86*:651.
12. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
13. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
14. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.
15. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Lowenstein Medium Base

Cat. No.	244420	Dehydrated – 500 g
	244410	Dehydrated – 2 kg

BBL™ Lowenstein-Jensen Medium

BS12 CMPH2 EP MCM9 SMWW

Cat. No.	220908	Prepared Slants (A Tubes) – Pkg. of 10*
	220909	Prepared Slants (A Tubes) – Ctn. of 100*
	221387	Prepared Slants (C Tubes) – Pkg. of 10*
	221388	Prepared Slants (C Tubes) – Ctn. of 100*
	221257	Prepared Deeps (A Tubes) – Pkg. of 10*
	221115	**Mycoflask**™ Bottle – Pkg. of 10*
	221116	**Mycoflask**™ Bottles – Ctn. of 100*
	295701	Bottles, 1 oz – Ctn. of 100*

BBL™ Lowenstein-Jensen Medium, Gruft

BS12 CMPH2 MCM9

Cat. No.	297608	Prepared Slants (A Tubes) – Pkg. of 10*
	297653	Prepared Slants (A Tubes) – Ctn. of 100*
	297211	Prepared Slants (C Tubes) – Pkg. of 10*
	297703	Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Lowenstein-Jensen Medium with Iron

Cat. No.	297206	Prepared Slants (C Tubes) – Pkg. of 10*

BBL™ Lowenstein-Jensen Medium with Pyruvic Acid

Cat. No.	297270	Prepared (Transgrow-style bottle) – Ctn. of 100*

BBL™ Lowenstein-Jensen Medium with 5% Sodium Chloride

Cat. No.	221896	Prepared Slants (C Tubes) – Pkg. of 10*

**Store at 2-8°C.*

Luria Agar Base, Miller • Luria Broth Base, Miller

Intended Use

Luria Agar Base, Miller and Luria Broth Base, Miller are used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures with or without added glucose.

Summary and Explanation

Luria Agar Base, Miller and Luria Broth Base, Miller are nutritionally rich media designed for growth of pure cultures of recombinant strains, based on the Luria agar and broth formulae described by Miller.[1] *E. coli* is grown to late log phase in LB Medium. Some plasmid vectors replicate to high copy numbers and do not require selective amplification. Some vectors do not replicate so freely and need to be selectively amplified. Chloramphenicol may be added to inhibit host synthesis and, as a result, prevent replication of the bacterial chromosome.[2]

Luria Agar Base, Miller and Luria Broth Base, Miller contain one-tenth and one-twentieth, respectively, the sodium chloride level of the LB Agar, Lennox and LB Agar, Miller formulations.[1-3] This allows the researcher to select the optimal salt concentration for a specific strain. The medium may be aseptically supplemented with glucose, if desired.

User Quality Control

Identity Specifications

Difco™ Luria Agar Base, Miller

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.05% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Very light amber, slightly opalescent.
Reaction of 3.05% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ Luria Broth Base, Miller

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	1.55% solution, soluble in purified water. Solution is very light to light amber, clear to very slightly opalescent.
Prepared Appearance:	Light to light amber, clear to very slightly opalescent.
Reaction of 1.55% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Luria Agar Base, Miller or Luria Broth Base, Miller

Prepare the medium with 10 mL sterile 20% glucose solution per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli (K802)	33526	10^2-3×10^2 (Agar)	Good
		10^2-10^3 (Broth)	Good

Principles of the Procedure

Peptone and yeast extract provide nitrogen, carbon, vitamins (including B vitamins) and certain trace elements. Sodium chloride provides essential ions. Agar is the solidifying agent.

Formulae

Difco™ Luria Agar Base, Miller

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Yeast Extract	5.0	g
Sodium Chloride	0.5	g
Agar	15.0	g

Difco™ Luria Broth Base, Miller

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ Luria Agar Base, Miller – 30.5 g;
   Difco™ Luria Broth Base, Miller – 15.5 g.
   Mix thoroughly.
2. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. If desired, aseptically add 10 mL of sterile 20% glucose solution and mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[1,2]

Expected Results
Growth is evident in the form of isolated colonies and/or a confluent lawn on the surface of the agar medium or the appearance of turbidity in the broth medium.

References

1. Miller. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. Lennox. 1955. Virology *1*:190.

Availability
Difco™ Luria Agar Base, Miller

Cat. No.	241320	Dehydrated – 500 g
	211829	Dehydrated – 2 kg

Difco™ Luria Broth Base, Miller

COMPF

Cat. No.	241420	Dehydrated – 500 g
	241410	Dehydrated – 2 kg

Lysine Assay Medium
(See Amino Acid Assay Media)

Lysine Decarboxylase Broth
(See Decarboxylase Differential Media)

Lysine Iron Agar

Intended Use
Lysine Iron Agar is used for the differentiation of enteric organisms based on their ability to decarboxylate or deaminate lysine and to form hydrogen sulfide.

Summary and Explanation
Edwards and Fife devised Lysine Iron Agar for the detection of *Salmonella enterica* subsp. *arizonae* (previously *Arizona arizonae*) cultures, especially those that ferment lactose rapidly.[1] This development followed closely the promulgation by Ewing and Edwards of a taxonomic scheme for the *Enterobacteriaceae* in which the principle division and groups within this family were defined and differentiation procedures described.[2] The various criteria for identification of cultures were summarized by Edwards and Ewing in their treatise on the *Enterobacteriaceae*.[3] However, the taxonomy of the *Enterobacteriaceae* has changed dramatically in recent years.[4-6]

Johnson et al. utilized Lysine Iron Agar and Kligler Iron Agar for primary differentiation of various groups of bacteria within the family *Enterobacteriaceae* and a combination of Lysine Iron Agar with Triple Sugar Iron Agar for identification of *Salmonella*, *Shigella* and *Arizona* group organisms from feces.[7]

Lysine Iron Agar aids in the differentiation of enteric bacilli on the basis of their ability to decarboxylate lysine, to deaminate lysine and to produce hydrogen sulfide. It is designed for use with other media (e.g., Triple Sugar Iron Agar) in appropriate identification schemes.

Principles of the Procedure
Dextrose serves as a source of fermentable carbohydrate. The pH indicator, bromcresol purple, is changed to a yellow color at or below pH 5.2 and is purple at or above pH 6.8.[8] Ferric ammonium citrate and sodium thiosulfate are indicators of hydrogen sulfide formation. Lysine is the substrate for use in detecting the enzymes, lysine decarboxylase and lysine deaminase.

Cultures of enteric bacilli that produce hydrogen sulfide cause blackening of the medium due to the production of ferrous sulfides. Those that produce lysine decarboxylase produce an alkaline reaction (purple color) or neutral reaction in the butt of the medium. Organisms that deaminate the lysine cause the development of a red slant over an acid butt. Gas may be formed but its formation is often irregular or suppressed.

Formulae
Difco™ Lysine Iron Agar

Approximate Formula* Per Liter		
Peptone	5.0	g
Yeast Extract	3.0	g
Dextrose	1.0	g
L-Lysine HCl	10.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Thiosulfate	0.04	g
Bromcresol Purple	0.02	g
Agar	15.0	g

BBL™ Lysine Iron Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	5.0	g
Yeast Extract	3.0	g
Dextrose	1.0	g
L-Lysine	10.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Thiosulfate	0.04	g
Bromcresol Purple	0.02	g
Agar	13.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Lysine Iron Agar

Dehydrated Appearance:	Beige to greenish beige, free flowing, homogeneous.
Solution:	3.45% solution, soluble in purified water upon boiling. Solution is reddish purple, slightly opalescent.
Prepared Appearance:	Purple, slightly opalescent.
Reaction of 3.45% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Lysine Iron Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	REACTION SLANT/BUTT	H_2S
Proteus mirabilis	25933	Good	Red/acid	–
Salmonella enterica subsp. *arizonae*	13314	Good	Alkaline/alkaline	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	Alkaline/alkaline	+
Shigella flexneri	12022	Good	Alkaline/acid	–

Alkaline = red purple, no change in color
Acid = yellow
Red = lysine deaminase
+ H_2S = black precipitate
– H_2S = no black precipitate

Identity Specifications

BBL™ Lysine Iron Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.3% solution, soluble in purified water upon boiling. Solution is medium to dark, rose purple, clear to slightly hazy.
Prepared Appearance:	Medium to dark, rose purple, clear to slightly hazy.
Reaction of 3.3% Solution at 25°C:	pH 6.7 ± 0.2

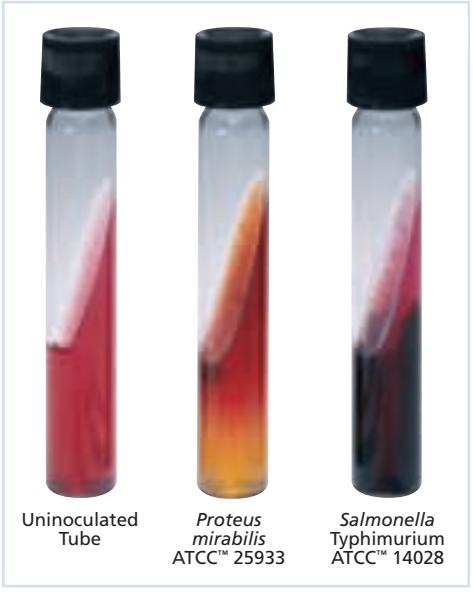
Cultural Response

BBL™ Lysine Iron Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	RECOVERY	REACTION SLANT/BUTT	H_2S
Citrobacter freundii	8454	Good	K/A, w/ or w/o gas	+
Escherichia coli	25922	Good	K/Weak K to K, w/ or w/o gas	–
Proteus vulgaris	9484	Good	R/A, w/ or w/o gas	–
Providencia rustigianii	13159	Good	R/A, w/ or w/o gas	–
Salmonella enterica subsp. *arizonae*	13314	Good	K /K, w/ or w/o gas	+
Salmonella enterica subsp. *enterica* serotype Paratyphi A	9150	Good	K/A, w/ or w/o gas	–
Salmonella enterica subsp. *enterica* serotype Typhi	19430	Good	K/K, w/ or w/o gas	+

A = Acid (yellow)
K = Alkaline (red purple, no change in color)
R = Red (lysine deaminase)
+ H_2S = black precipitate
– H_2S = no black precipitate



Uninoculated Tube | *Proteus mirabilis* ATCC™ 25933 | *Salmonella* Typhimurium ATCC™ 14028

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Lysine Iron Agar – 34.5 g;
   BBL™ Lysine Iron Agar – 33 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 12 minutes.
4. Cool tubes in a slanted position to form slants with deep butts.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Using an inoculating needle, stab the butt twice then streak the slant with growth from a pure culture. Incubate tubes with loosened caps for 18-48 hours at 35 ± 2°C in an aerobic atmosphere.

Triple Sugar Iron Agar slants should be inoculated in parallel unless results from this medium have already been obtained to distinguish coliforms from *Shigella*, for example.

Expected Results

Lysine decarboxylation is detected in the butt by an alkaline (purple) reaction. Lysine deamination is detected by a red slant. Hydrogen sulfide production is detected by the formation of a black precipitate. A negative reaction (purple slant and yellow butt) indicates fermentation of dextrose only.[8]

Hydrogen sulfide may not be detected in this medium by organisms that are negative for lysine decarboxylase activity since acid production in the butt may suppress its formation.[8] For this reason H_2S-producing *Proteus* species do not blacken this medium.[8]

References

1. Edwards and Fife. 1961. Appl. Microbiol. 9:478.
2. Ewing and Edwards. 1960. Int. Bull. Bacteriol. Nomencl. Taxon. *10*:1.
3. Edwards and Ewing. 1962. Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis, Minn.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
7. Johnson, Kunz, Barron and Ewing. 1966. Appl. Microbiol. *14*:212.
8. MacFaddin. 1985. Media for isola on-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore Md.

Availability

Difco™ Lysine Iron Agar

AOAC | BAM | CCAM | CMPH2 | COMPF | MCM9 | SMD | SMWW | USDA

Cat. No. 284920 Dehydrated – 500 g

BBL™ Lysine Iron Agar

AOAC | BAM | CCAM | CMPH2 | COMPF | MCM9 | SMD | SMWW | USDA

Cat. No. 211363 Dehydrated – 500 g
220952 Prepared Slants – Pkg. of 10*
220953 Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

Lysozyme Broth • Lysozyme Control

Intended Use

Lysozyme Broth and Lysozyme Control are used in the identification of *Nocardia* species based on resistance to lysozyme.

Summary and Explanation

Nocardiosis is a disease of man and animals caused by *Nocardia* species. The disease may resemble tuberculosis if the organism is inhaled or produce granulomatous abscesses if introduced into tissues at the time of the injury.

Lysozyme Broth is used with Lysozyme Control to identify *Nocardia* by determining resistance to the enzyme lysozyme, indicated by the presence of growth.[2] *Streptomyces* species, which resemble *Nocardia* morphologically, are inhibited by lysozyme.[3]

Principles of the Procedure

Enzymatic digest of casein and beef extract provide amino acids and other nitrogenous substances. Glycerol is a source of energy.

Lysozyme is a muramidase that lyses some gram-positive bacteria. *Nocardia* species are resistant to lysozyme, exhibiting growth in Lysozyme Broth and in the control medium, while *Streptomyces* species are susceptible and growth is inhibited.[2] The control medium contains the same nutrients as lysozyme broth, but without the enzyme to demonstrate that the test organism is capable of growth in the medium.

Procedure

The organism to be tested should be isolated in pure culture, Gram stained and examined to determine that morphology is appropriate for *Nocardia* species.

Using a sterile inoculating loop or needle, inoculate the Lysozyme Broth and Lysozyme Control with fragments of the culture being tested. Inoculate a control set of broths using a known *Streptomyces* species. The control set indicates whether the lysozyme is active. Incubate the tubes at 25°C for up to 7 days.

Expected Results

Nocardia species exhibit growth in both media, indicating resistance to lysozyme. If growth occurs in the control tube and not in the Lysozyme Broth, the organism is probably not a *Nocardia* species. The test is inconclusive if neither tube shows growth.

References

1. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994. U.S. Government Printing Office, Washington, D.C.
2. Haley and Callaway. 1978. Laboratory methods for medical mycology, 4th ed. Center for Disease Control, Atlanta, Ga.
3. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed., Lippincott-Raven Publishers, Philadelphia, Pa.

Availability

BBL™ Lysozyme Broth

Cat. No. 297231 Prepared Tubes (K Tubes), 5 mL – Pkg. of 10*

BBL™ Lysozyme Control

Cat. No. 297232 Prepared Tubes (K Tubes), 5 mL – Pkg. of 10*

**Store at 2-8°C.*

M9 Minimal Salts, 5×

Intended Use

M9 Minimal Salts, 5× is used in preparing M9 Minimal Medium which is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

M9 Minimal Salts, 5× is a 5× concentrate that is diluted to a 1× concentration and supplemented with an appropriate carbon and energy source, such as dextrose, to provide a minimal, chemically defined medium. The medium will support the growth of "wild-type" strains of *E. coli*. M9 Minimal Salts is useful for maintaining positive selection pressure on plasmids coding for the ability to produce essential substances such as amino acids or vitamins. M9 Minimal Medium is also used to maintain stocks of F´-containing bacteria for use with M13. The medium can be supplemented with specific amino acids or other metabolites, allowing for selection of specific auxotrophs.

Principles of the Procedure

Sodium phosphate and potassium phosphate are present as buffering agents. Ammonium chloride is a source of nitrogen for cellular systems. Sodium chloride provides essential ions. Glucose may be added as a source of carbohydrate. Supplementing the medium with magnesium and calcium increases the growth of recombinants.

Formula

Difco™ M9 Minimal Salts, 5×

Approximate Formula* Per Liter		
Disodium Phosphate (anhydrous)	33.9	g
Monopotassium Phosphate	15.0	g
Sodium Chloride	2.5	g
Ammonium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 56.4 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. To prepare M9 Minimal Salts Medium, add 200 mL sterile M9 Minimal Salts, 5× to 750 mL sterile purified water cooled to 45-50°C, adjusting the final volume to 1 liter.
4. Aseptically add 20 mL filter-sterilized 20% glucose solution, 2 mL sterile 1.0 M $MgSO_4$ solution and, if desired, 0.1 mL sterile 1.0 M $CaCl_2$ solution. Mix well.
5. If desired, supplement with amino acids, as appropriate.
6. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ M9 Minimal Salts, 5×

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	5.64% solution, soluble in purified water. Solution is colorless, clear, no significant precipitate.
Prepared Appearance:	Colorless, clear, no siginficant precipitate.
Reaction of 5.64% Solution (5× concentrate) at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ M9 Minimal Salts, 5×

Prepare the medium and dilute to 1x. Supplement with glucose per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli (Strain B)	23226	30-300	Good to excellent
Escherichia coli (JM103)	39403	30-300	Good to excellent

Procedure

Consult appropriate references for recommended test procedures.[1,2]

Expected Results

Growth should be evident by the appearance of turbidity.

References

1. Davis, Dibner and Battey. 1986. Basic methods in molecular biology. Elsevier, New York, N.Y.
2. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Availability

Difco™ M9 Minimal Salts, 5×

Cat. No. 248510 Dehydrated – 500 g

M17 Agar • M17 Broth

Intended Use

M17 Agar is used for isolating and enumerating lactic streptococci in yogurt, cheese starters and other dairy products.

M17 Broth is used for isolating lactic streptococci from yogurt, cheese starters and other dairy products.

Summary and Explanation

Lactic streptococci are acid-producing bacteria. They are nutritionally fastidious and require complex culture media for optimum growth. One study showed that in a synthetic medium, all strains had an obligate requirement for at least

User Quality Control

Identity Specifications

Difco™ M17 Agar

Dehydrated Appearance:	Beige to medium tan, free-flowing, homogeneous.
Solution:	48.25 g soluble in 950 mL purified water upon boiling. Solution is light-medium to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, very slightly to slightly opalescent, no significant precipitate.
Reaction of 48.25 g/950 mL at 25°C:	pH 6.9 ± 0.2

Difco™ M17 Broth

Dehydrated Appearance:	Beige to medium tan, free-flowing, homogeneous.
Solution:	37.25 g soluble in 950 mL purified water upon boiling. Solution is light-medium to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light medium to medium amber, clear to very slightly opalescent, no significant precipitate.
Reaction of 37.25 g/950 mL at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ M17 Agar or M17 Broth

Prepare the medium per label directions with the addition of lactose. Inoculate and incubate for 40-48 hours; *Lactococcus lactis* subsp. *cremoris* at 30 ± 2°C and the remaining organisms at 35 ± 2°C.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus delbrueckii subsp. *bulgaricus*	11842	10^2-10^3	None to poor
Lactococcus lactis subsp. *cremoris*	19257	10^2-10^3	Good
Lactococcus lactis subsp. *cremoris*	9625	10^2-10^3	Good
Streptococcus thermophilus	19258	10^2-10^3	Good

six amino acids and three vitamins.[1] These homofermentative lactic streptococci produce large amounts of acid and, in a culture medium without an adequate buffering system, the pH decreases and adversely affects growth. Lowrie and Pearce[2] developed M16 Medium but it lacked a strong buffering system. Terzaghi and Sandine[3] worked with M16 Medium and demonstrated that the rapid drop in pH that accompanies lactic streptococcal growth can adversely affect colony size and phage plaque formation. They modified M16 Medium using disodium-β-glycerophosphate as a buffer and called it M17.

Shankar and Davies[4] found that disodium-β-glycerophosphate in M17 Broth suppressed *Lactobacillus bulgaricus* and selectively isolated *Streptococcus thermophilus* from yogurt. Similar results were achieved using M17 Broth solidified with agar. The International Dairy Federation recommends M17 Agar for isolating *S. thermophilus* from yogurt.[5] M17 Agar is a standard methods medium for isolating lactic streptococci.[6]

Principles of the Procedure

M17 Agar and M17 Broth contain peptones and meat derivatives as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Disodium-β-glycerophosphate buffers the medium as acid is produced from fermentation of lactose. Ascorbic acid stimulates growth of lactic streptococci. Magnesium sulfate provides essential ions for growth. Agar is the solidifying agent in M17 Agar.

Formulae

Difco™ M17 Agar

Approximate Formula* Per 950 mL

Pancreatic Digest of Casein	5.0	g
Soy Peptone	5.0	g
Beef Extract	5.0	g
Yeast Extract	2.5	g
Ascorbic Acid	0.5	g
Magnesium Sulfate	0.25	g
Disodium-β-glycerophosphate	19.0	g
Agar	11.0	g

Difco™ M17 Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 950 mL of purified water.
   Difco™ M17 Agar – 48.25 g;
   Difco™ M17 Broth – 37.25 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50°C.
4. Add 50 mL sterile 10% lactose solution and mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Reiter and Oram. 1962. J. Dairy Res. *29*:63.
2. Lowrie and Pearce. 1971. J. Dairy Sci. Technol. *6*:166.
3. Terzaghi and Sandine. 1975. Appl. Microbiol. *29*:807.
4. Shankar and Davies. 1977. J. Soc. Dairy Tech. *30*:28.
5. International Dairy Federation. 1981. Identification and enumeration of microorganisms in fermented milks. Joint IDF/ISO/AOAC Group E44.
6. Richter and Vedamuthu 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ M17 Agar

COMPF ISO

Cat. No. 218571 Dehydrated – 500 g

Difco™ M17 Broth

Cat. No. 218561 Dehydrated – 500 g

Bacto™ M Broth

Intended Use

Bacto™ M Broth is used for cultivating *Salmonella* in foods and feeds by the accelerated enrichment serology (ES) procedure.

Summary and Explanation

M Broth, prepared according to the formula of Sperber and Diebel,[1] contains all the nutrients necessary for good growth and flagella development of *Salmonella*.

Fantasia, Sperber and Deibel[2] compared the enrichment serology (ES) procedure with the traditional procedure that was outlined in the *Bacteriological Analytical Manual*[3] (BAM) and reported excellent agreement between the two. They found the ES procedure not only to be faster and less complicated but also as accurate and sensitive as the BAM procedure.

M Broth also conforms to the testing standards recommended by the *Compendium of Methods for the Microbiological Examination of Foods*[4] (APHA) for the isolation and identification of foodborne *Salmonella*.

Monoclonal enzyme immunoassay (EIA) methods have been described in *Official Methods of Analysis of AOAC International*[5] using M Broth. These methods are screening procedures for the presence of *Salmonella* and positive results must be confirmed by culture.

Principles of the Procedure

Yeast extract is a source of B-complex vitamins. Peptone provides organic nitrogen. D-Mannose and sodium citrate are fermentation energy sources. Mannose prevents fimbrial agglutination.[1] Sodium chloride helps maintain osmotic equilibrium, while dipotassium phosphate acts as a buffer. The inorganic salts stimulate bacterial growth. Polysorbate 80 is a surfactant and dispersing agent.

Formula

Bacto™ M Broth

Approximate Formula* Per Liter		
Yeast Extract	5.0	g
Pancreatic Digest of Casein	12.5	g
D-Mannose	2.0	g
Sodium Citrate	5.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	5.0	g
Manganese Chloride	0.14	g
Magnesium Sulfate	0.8	g
Ferrous Sulfate	0.04	g
Polysorbate 80	0.75	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Bacto™ M Broth

Dehydrated appearance:	Beige, homogeneous with a tendency to clump.
Solution:	3.62% solution, soluble in purified water upon boiling. Solution is light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 3.62% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Bacto™ M Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Salmonella enterica subsp. *enterica* serotype Choleraesuis var. Kunzendorf	12011	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good

Uninoculated Tube

Salmonella Choleraesuis ATCC™ 12011

Procedure

1. Prepare a 10% suspension of the test sample in Lactose Broth. Incubate at 35 ± 2°C for 18-24 hours.
2. Transfer 1 mL of the above preenrichment culture to 9 mL of Selenite Cystine Broth and 1 mL to 9 mL of Tetrathionate Broth. Incubate both enrichment media at 35 ± 2°C for 24 hours.
3. Inoculate one 10 mL tube of M Broth, tempered to 35°C, with one drop from each of the above cultures. Incubate at 35 ± 2°C for 6-8 hours.
4. Prepare a formalin-salt solution by adding 4.2 g of NaCl and 3 mL of formalin to 100 mL of purified water. Place one drop in each of two Kahn tubes.
5. Carefully insert a pipette about 1 inch below the surface of the M Broth culture and transfer 0.85 mL of culture to each of the above Kahn tubes containing formalin-salt solution.
6. Prepare a pooled antiserum by combining together 0.5 mL each of rehydrated Salmonella H Antiserum Poly D and Salmonella H Antiserum z_6 (Salmonella H Antisera Spicer-Edwards Set) in 11.5 mL of 0.85% NaCl.
7. Add 0.1 mL pooled Salmonella H Antiserum to one of the Kahn tubes (above). Add 0.1 mL 0.85% NaCl solution to the other tube. Shake the tubes gently. Incubate in a 50°C water bath for 1.5 hours.

Expected Results

Agglutination in the Kahn tube containing antiserum indicates the presence of *Salmonella*. Agglutination in the Kahn tube containing 0.85% NaCl solution (control tube) indicates a rough culture which should be streaked for isolation, passed through Motility GI Medium to enhance flagella and then retested with pooled antiserum.

Alternative Testing Procedures

Refer to *Official Methods of Analysis of AOAC International*[5] for screening procedures using enzyme immunoassay or DNA hybridization to detect *Salmonella* antigen in test samples.

References

1. Sperber and Deibel. 1969. Appl. Microbiol. *17*:533.
2. Fantasia, Sperber and Deibel. 1969. Appl. Microbiol. *17*:540.
3. Bacteriological Analytical Manual, 2nd ed. 1969. USDHEW, Washington, D.C.
4. Andrews, Flowers, Silliker and Bailey. 2001. *In* Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, Md.

Availability

Bacto™ M Broth

AOAC COMPF USDA

Cat. No.	294020	Dehydrated – 500 g*
	294010	Dehydrated – 2 kg*

**Store at 2-8°C.*

MI Agar

Intended Use

MI Agar* is a chromogenic/fluorogenic medium used to detect and enumerate *Escherichia coli* and total coliforms in drinking water by the membrane filtration technique. It conforms with the U.S. Environmental Protection Agency (USEPA) Approved Method 1604: *Total Coliforms and* Escherichia coli *in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)*.

** U.S. Patent No. 6,063,590. Manufactured under license. Commercialization of dehydrated culture medium as prepared medium is prohibited.*

Summary and Explanation

Coliform bacteria are species that inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation and water. They are usually found in fecally-polluted water and are often associated with disease outbreaks. Although these bacteria are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of other pathogens. *E. coli* is one species in this group of coliform bacteria. Since it is always found in feces, it is a more direct indicator of fecal contamination and the possible presence of enteric pathogens.

Chromogens or fluorogens have been used for many years to detect and identify total coliforms (TC) and *E. coli*. Some methods use liquid media in a multiple-tube-fermentation (MTF) test, a presence-absence (PA) format or other tube tests. Agar media are also used for direct plating or membrane filtration (MF) technology. However, standard MF technology for the detection of TC and fecal coliforms requires the use of several different types of media and two different incubation temperatures.[1]

The newest technology developed by the USEPA for testing drinking water is a single membrane filtration technique where no membrane filter transfers are required.[1-4] The medium is named after the two enzyme substrates included in the formulation: a fluorogen, 4-Methylumbelliferyl-β-D-galactopyranoside (MUGal) and a chromogen, Indoxyl-β-D-glucuronide (IBDG). MI Agar can simultaneously detect and enumerate both TC and *E. coli* in water samples in 24 hours or less based on their specific enzyme activities. MI Agar detects the presence of the bacterial enzymes β-galactosidase and β-glucuronidase produced by TC and *E. coli*, respectively.

MI Agar is approved for use by certified drinking water laboratories for microbial analysis of potable water. Other uses include recreational, surface or marine water, bottled water, groundwater, well water, treatment plant effluents, water from drinking water distribution line, drinking water source water and possibly foods.[5]

As referenced in USEPA method 1604, this method has a detection limit of one *E. coli* and/or one total coliform per sample volume or dilution tested.[5] The false-positive and false negative rates for *E. coli* are both 4.3%.[5] Specificity for *E. coli* is 95.7%

User Quality Control

Identity Specifications

Difco™ MI Agar

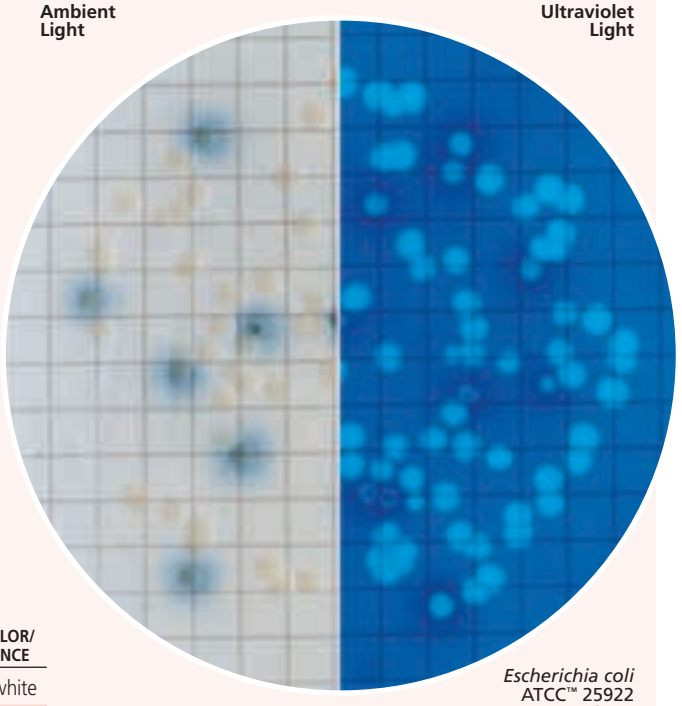
Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.65% solution, soluble in purified water upon boiling. Solution is light to medium tan, very slightly to slightly opalescent.
Prepared Appearance:	Light tan, clear to very slightly opalescent. Upon removal from 2-8°C storage, plates may exhibit a crystal precipitate that disappears upon warming to room temperature. This is a typical characteristic of the medium and is acceptable.
Reaction of 3.65% Solution at 25°C:	pH 6.95 ± 0.2

Cultural Response

Difco™ MI Agar

Prepare the medium per label directions. Inoculate using the membrane filtration technique and incubate at 35 ± 2°C for 20-24 hours. Count all blue or indigo colonies under ambient light. Expose MI Agar plates to long-wave ultraviolet light (366 nm) and count all fluorescent colonies.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR/ FLUORESCENCE
Enterobacter aerogenes	13048	20-80	Good	Tan / blue-white
Escherichia coli	25922	20-80	Good	Blue / blue-green
Proteus mirabilis	43071	20-80	Good	Tan / none
Pseudomonas aeruginosa	27853	20-80	Marked to complete inhibition	Tan / none



Escherichia coli ATCC™ 25922 and Coliform

and for total coliforms is 93.1%.[5] The single lab recovery of *E. coli* is 97.9% of the heterotrophic plate count (pour plate) and 115% of the R2A spread plate count.[5] For *Klebsiella pneumoniae* and *Enterobacter aerogenes*, recoveries are 87.5% and 85.7% of the heterotrophic plate count and 89.3% and 85.8% of the R2A spread plate method, respectively.[5]

Principles of the Procedure

MI Agar contains peptone as a source of nitrogen, carbon and amino acids. Yeast extract provides trace elements, vitamins and amino acids. Lactose is a fermentable carbohydrate and carbon source. Sodium chloride maintains osmotic equilibrium. Monopotassium and dipotassium phosphates offer buffering capabilities. Sodium lauryl sulfate and sodium desoxycholate are selective against gram-positive bacteria. *E. coli* that produce the enzyme β-D-glucuronidase cleave the chromogen indoxyl-β-D-glucuronide (IBDG) to form a blue- or indigo-colored compound. The β-galactosidase produced by total coliforms cleaves the fluorogen 4-methylumbelliferyl-β-D-galactopyranoside (MUGal), producing 4-methylumbelliferone, a fluorescent compound when exposed to long-wave UV light (366 nm). Agar is the solidifying agent. Celfsulodin is added to inhibit gram-positive bacteria and some non-coliform gram-negative bacteria that may cause false positives.

Formula

Difco™ MI Agar

Approximate Formula* Per Liter

Proteose Peptone No. 3	5.0	g
Yeast Extract	3.0	g
D-Lactose	1.0	g
MUGal (4-Methylumbelliferyl-β-D-galactopyranoside)	0.1	g
Indoxyl-β-D-glucuronide (IBDG)	0.32	g
Sodium Chloride	7.5	g
Dipotassium Phosphate	3.3	g
Monopotassium Phosphate	1.0	g
Sodium Lauryl Sulfate	0.2	g
Sodium Desoxycholate	0.1	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes and cool in a 50°C water bath.
4. Add 5 mL of a freshly prepared 1 mg/mL filter-sterilized solution of cefsulodin per liter of tempered agar medium (final concentration of 5 µg/mL).
5. Dispense 5 mL amounts into 9 × 50 mm or 15 × 60 mm plates and allow to solidify.
6. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Upon removal from 2-8°C storage, plates may exhibit a crystal precipitate that disappears upon warming to room temperature. This is a typical characteristic of the medium and is acceptable.

Procedure

1. Collect and prepare water samples in accordance to recommended guidelines.[6,7]
2. Test recommended sample volumes following the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.[6] For drinking water, test 100 mL samples. For other samples, select sample volumes to produce 20-80 colonies of the target organism on the membrane filter. With water of good quality, the occurrence of coliforms generally will be minimal. Therefore, count all coliform colonies (disregarding the lower limit of 20 cited above) and use the formula given below to obtain coliform density.[6]
3. After sample has been filtered, aseptically remove membrane filter from filter base and roll it onto MI Agar to avoid the formation of bubbles between the membrane and the agar surface.
4. Invert inoculated plates and incubate at 35 ± 0.5°C for 20-24 hours.
5. After incubation, count and record the number of blue or indigo colonies under normal/ambient light to obtain *E. coli* count.
6. Expose each MI Agar plate to long-wave ultraviolet light (366 nm), and count and record all fluorescent colonies plus all blue, non-fluorescent colonies to obtain a total coliform count.

For drinking water, calculate and report the number of *E. coli* and Total Coliform colonies per 100 mL of sample using the formula:

$$\textit{E. coli}\ /100\ \text{mL} = \frac{\text{Number of blue colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

$$\text{Total Coliforms}\ /100\ \text{mL} = \frac{\text{Number of FC* + Number of blue NFC**}}{\text{Volume of sample filtered (mL)}} \times 100$$

**FC = Fluorescent colonies*

***NFC = Non-fluorescent colonies*

Expected Results

E. coli produces blue or indigo colonies under normal/ambient light. Total coliforms produce blue/green fluorescent colonies (*E. coli*), blue/white fluorescent colonies (total coliforms other than *E. coli*) and blue/green colonies with fluorescent edges (*E. coli*). In addition, any blue, non-fluorescent colonies (also *E. coli*) found should be added to the total coliform count.[1]

Refer to the USEPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.[8]

Limitations of the Procedure

1. For water samples other than drinking water, choose a water sample size that will result in 20-80 colonies per filter. The ideal volume for *E. coli* enumeration may not be optimum for total coliform enumeration, and vise versa. However, since blue *E. coli* colonies were found to be clearly visible on a total coliform background that was too numerous to count, this should be of minor importance for drinking water compliance purposes, and multiple volumes or dilutions of other water types should provide accurate enumeration.[1]
2. Water samples containing colloidal or suspended particulate material can clog the membrane filter, preventing adequate filtration or causing the spread of bacterial colonies. This could interfere with identification of the target colonies. However, the blue *E. coli* colonies can often be counted on plates with heavy particulate matter or high concentrations of total bacteria.[1]
3. The presence of some lateral diffusion of blue color away from the target *E. coli* colonies can affect enumeration and colony picking on plates with high concentrations of *E. coli* . This problem should not affect filters with low counts, such as those obtained with drinking water or properly diluted samples.[1]
4. Colonies that are tiny, flat or peaked pinpoint blue, with a size of less than 0.5 mm in diameter on filters containing less than 200 colonies may be due to species other than *E. coli* . These colonies occur occasionally in low numbers and should be excluded from the count of *E. coli* colonies, which are usually much larger in size (1-3 mm in diameter). The small colonies have never been observed in the absence of typical *E. coli* but, if they should occur, the sample should not be considered *E. coli*-positive unless at least one colony has been verified by another method.[1]
5. Bright green, fluorescent, non-blue colonies, observed along with the typical blue/white or blue/green fluorescent TC colonies, may be species other than coliforms. These colonies generally occur in low numbers (≤ 5%) and can usually be distinguished from the total coliforms. An increase in the number of bright green colonies may indicate an unusual sample population or a breakdown of the cefsulodin in the medium.[1]
6. Minimize the exposure of MI Agar to light before and during incubation, as light may destroy the chromogen.

References

1. Brenner, Rankin, Roybal, Stelma, Scarpino and Dufour. 1993. Appl. Environ. Microbiol. 59:3534.
2. Brenner, Rankin and Sivaganesan. 1996. J. Microbiol. Methods. 27:111.
3. Brenner, Rankin, Sivaganesan and Scarpino. 1996. Appl. Environ. Microbiol. 62:203.
4. U.S. Environmental Protection Agency. 2000. Membrane filter method for the simultaneous detection of total coliforms and *Escherichia coli* in drinking water. Publication EPA 600-R-00-013. Office of Research and Development, USEPA, Cincinnati, Ohio.
5. U.S. Environmental Protection Agency. 2002. Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI medium). Publication EPA-821-R-02-024. USEPA Office of Water (4303T), Washington, DC.
6. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
7. ASTM International. 2007. 2007 ASTM Annual book of standards 11: Water and environmental technology. ASTM International, West Conshohocken, Pa.
8. Bordner, Winter and Scarpino (ed.). 1978. Microbiological methods for monitoring the environment: water and wastes. Publication EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Availability

Difco™ MI Agar

EPA

Cat. No.	214882	Dehydrated – 100 g
	214883	Dehydrated – 500 g

BBL™ MI Agar

EPA

Cat. No.	214986	Prepared Plates – Pkg. of 20*
	214985	Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*

MIL Medium

Intended Use

MIL Medium is used for differentiating *Enterobacteriaceae* based on motility, lysine decarboxylation, lysine deamination and indole production.

Summary and Explanation

MIL (Motility-Indole-Lysine) Medium, prepared according to the formula of Reller and Mirrett,[1] is a single culture medium that provides four differentiating biochemical reactions. When used in conjunction with Triple Sugar Iron Agar (TSI) and Urea Agar, as many as nine reactions are provided. This combination enables reliable initial identification of *Enterobacteriaceae*.[2,3] Extensive testing of 890 enteric cultures by Reller and Mirrett[1] gave essentially the same results with MIL Medium as with the standard motility, indole and lysine decarboxylase (Moeller) test media.

Principles of the Procedure

Peptones provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Yeast extract provides vitamins and cofactors required for growth. Lysine hydrochloride is present as a substrate to detect lysine decarboxylase or lysine deaminase activity. Dextrose is an energy source. Ferric ammonium citrate is an H_2S indicator. Bromcresol purple is a pH indicator. Agar is the solidifying agent.

Formula

Difco™ MIL Medium

Approximate Formula* Per Liter

Peptone	10.0	g
Pancreatic Digest of Casein	10.0	g
Yeast Extract	3.0	g
L-Lysine HCl	10.0	g
Dextrose	1.0	g
Ferric Ammonium Citrate	0.5	g
Bromcresol Purple	0.02	g
Agar	2.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ MIL Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.65% solution, soluble in purified water upon boiling. Solution is reddish-purple, clear.
Prepared Appearance:	Reddish purple, clear, semisolid.
Reaction of 3.65% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Difco™ MIL Medium

Prepare the medium per label directions. Stab inoculate using fresh cultures and incubate at 35 ± 2°C for 18-24 hours. After reading the lysine decarboxylase, motility and lysine deaminase reactions, add Indole Reagent Kovacs to determine the indole reaction.

ORGANISM	ATCC™	LYSINE DECARBOXYLASE	MOTILITY	LYSINE DEAMINASE	INDOLE PRODUCTION
Escherichia coli	25922	+	+	–	+
Providencia alcalifaciens	9886	–	+	+	–
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	+	+	–	–
Shigella flexneri	12022	–	–	–	–

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Shigella flexneri* ATCC™ 12022

All with Indole Reagent

Procedure

1. Using an inoculating needle, stab tubes (13 × 100 mm screw-capped tubes containing 5 mL) with growth from an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C for 18-24 hours.
3. After incubation, examine tubes for evidence of lysine deaminase, motility, lysine decarboxylase reactions and, after addition of Indole Reagent Kovacs, indole production.

Expected Results

Lysine deaminase is indicated by a red or red-brown color in the top centimeter of the medium.

Motility is indicated by a clouding of the medium or by growth extending from the inoculation line.

Lysine decarboxylase is indicated by a purple color throughout the medium. This color may vary in intensity and may be bleached out to a pale light color due to reduction of the indicator. Lysine-negative cultures produce a yellow medium that may be purple or red on the top. Tubes that show a purple reaction with a red color on top should be incubated for a longer period of time.

After examining the medium for lysine deaminase, motility and lysine decarboxylase reactions, add 3 or 4 drops of Indole Reagent Kovacs (Cat. No. 261185) to the top of each tube. The appearance of a pink to red color in the reagent is interpreted as a positive indole test.

Positive and negative reactions are based on 90% or more occurrences. When an aberrant reaction occurs, subcultures should be plated on differential media to ensure the purity of the culture.

Limitations of the Procedure

1. Do not add Indole Reagent Kovacs until the final lysine deaminase, lysine decarboxylase and motility results have been interpreted.
2. Occasionally, the indole test produces false-negative or falsely weak reactions.[4]

References

1. Reller and Mirrett. 1975. J. Clin. Microbiol. 2:247.
2. Murray, Baron, Jorgensen, Landry and Pfaller. (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Forbes, Sahm and Weissfeld. 2007. Bailey and Scott's diagnostic microbiology, 12th ed. Mosby Inc., St. Louis, Mo.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ MIL Medium

Cat. No. 218041 Dehydrated – 500 g

Difco™/BBL™ Indole Reagent

Cat. No. 261185 Droppers, 0.5 mL – Ctn. of 50

MIO Medium • Motility Indole Ornithine Medium

Intended Use

Motility Indole Ornithine (MIO) Medium is used to demonstrate motility, indole production and ornithine decarboxylase activity for the differentiation of *Enterobacteriaceae*.

Summary and Explanation

MIO Medium was formulated by Ederer and Clark[1] and Oberhofer and Hajkowski[2] for detection of motility, indole and ornithine decarboxylase production in one tube as an aid in the identification of members of the *Enterobacteriaceae* family.

Principles of the Procedure

Peptones, yeast extract and dextrose provide amino acids and other nitrogenous and carbonaceous substances, vitamins and minerals essential for bacterial metabolism. Motility can be read because of the semi-solid consistency of the medium. Organisms that possess the enzyme "tryptophanase" degrade the amino acid tryptophan to indolepyruvic acid, from which indole can be formed through deamination.[3] When ornithine decarboxylase is present, the ornithine is decarboxylated to putrescine which causes a rise in the pH and corresponding color change of the bromcresol purple from yellow to purple.

Formula

Difco™ MIO Medium

Approximate Formula* Per Liter		
Yeast Extract	3.0	g
Peptone	10.0	g
Tryptone	10.0	g
L-Ornithine HCl	5.0	g
Dextrose	1.0	g
Agar	2.0	g
Bromcresol Purple	0.02	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To prepare the stored medium for use in motility studies, loosen caps, heat the medium to boiling and cool to room temperature prior to inoculation. Inoculate tubes of medium by a single stab to 1/4 inch from the bottom of the tube using growth from a

User Quality Control

Identity Specifications

Difco™ MIO Medium

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water upon boiling. Solution is purple, clear to slightly opalescent.
Prepared Appearance:	Purple, slightly opalescent, semi-solid.
Reaction of 3.1% Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

Difco™ MIO Medium

Prepare the medium per label directions. Inoculate with fresh cultures using an inoculating needle and incubate with caps loosened at 35 ± 2°C for 24-48 hours. Detect the presence of indole by the addition of 3-4 drops of Kovacs' Reagent.

ORGANISM	ATCC™	MOTILITY	INDOLE	ORNITHINE
Enterobacter aerogenes	13048	+	–	+
Escherichia coli	25922	+	+	+
Klebsiella pneumoniae subsp. *pneumoniae*	13883	–	–	–
Proteus mirabilis	25933	+	–	+

Uninoculated Tube | *Enterobacter aerogenes* ATCC™ 13048 | *Escherichia coli* ATCC™ 25922

primary isolation plate or other pure culture. Incubate all tubes for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Read motility and decarboxylase activity prior to the addition of the reagent for the detection of indole production.

1. Motility is indicated by growth extending from the line of inoculation. Nonmotile organisms grow only along the line of inoculation.
2. Decarboxylation of ornithine is indicated by the development of a turbid purple to a faded yellow-purple color. A negative reaction is indicated by a yellow color.
3. Indole production is indicated by the formation of a pink to red color after the addition of three or four drops of Kovacs' reagent to the surface of the medium and gentle shaking. A negative reaction is indicated by the development of a yellow color.

Refer to appropriate texts for typical reactions produced by various members of the *Enterobacteriaceae*.[4-6]

References

1. Ederer and Clark. 1970. Appl. Microbiol. 2:849.
2. Oberhofer and Hajkowski. 1970. Am. J. Clin. Pathol. *54*:720.
3. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ MIO Medium

BAM

Cat. No. 273520 Dehydrated – 500 g

BBL™ Motility Indole Ornithine Medium

BAM

Cat. No. 221517 Prepared Deeps (K Tubes), 5 mL – Pkg. of 10*
221518 Prepared Deeps (K Tubes), 5 mL – Ctn. of 100*

*Store at 2-8°C.

MR-VP Medium • MR-VP Broth

Intended Use

MR-VP Medium and MR-VP Broth (Methyl Red-Voges Proskauer Medium/Broth, also known as Buffered Peptone-Glucose Broth) are used for the differentiation of bacteria by means of the methyl red and Voges-Proskauer reactions.

Summary and Explanation

Voges and Proskauer, in the latter part of the 19th century, reported the initial observations regarding the production of a red color after the addition of potassium hydroxide to specific culture media in which various organisms had grown.[1]

Clark and Lubs,[2] in 1915, found that the addition of methyl red to cultures of *Escherichia coli* resulted in a red color due to the high acidity produced during the fermentation of dextrose. The smaller amount of acid produced by *Klebsiella pneumoniae* and *Enterobacter aerogenes* is converted to acetoin resulting in an alkaline reaction (negative methyl red test).

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ MR-VP Medium

Dehydrated Appearance:	Very light to light beige, free-flowing, homogeneous.
Solution:	1.7% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 1.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ MR-VP Medium

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	RECOVERY	METHYL RED	VOGES-PROSKAUER
Enterbacter aerogenes	13048	Good	– (yellow)	+ (red)
Escherichia coli	25922	Good	+ (red)	– (no change)
Klebsiella pneumoniae subsp. *pneumoniae*	23357	Good	– (yellow)	+ (red)

Identity Specifications

BBL™ MR-VP Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.7% solution, soluble in purified water. Solution is pale to light, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Pale to light, yellow to tan, clear to slightly hazy.
Reaction of 1.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

BBL™ MR-VP Broth

Prepare the medium per label directions. Inoculate two sets of tubes (3 mL, Voges-Proskauer and 5 mL, Methyl Red) with fresh cultures and incubate at 35 ± 2°C for 48 hours (3 mL) and 5 days (5 mL).

ORGANISM	ATCC™	RECOVERY	METHYL RED	VOGES-PROSKAUER
Citrobacter freundii	8454	Good	+ (red)	– (no change)
Enterbacter aerogenes	13048	Good	– (yellow)	+ (red)
Escherichia coli	25922	Good	+ (red)	– (no change)
Klebsiella pneumoniae subsp. *pneumoniae*	33495	Good	– (yellow)	+ (red)

In the Voges-Proskauer test, Reagent A (5% [w/v] alpha-naphthol in absolute alcohol) contains a catalyst enhancing the formation of specific metabolic products that form a red complex upon the addition of Reagent B (40% [w/v] potassium hydroxide in purified water).

MR-VP Medium/Broth was developed to enable both the MR and the VP tests to be performed in the same medium, although in different tubes or on aliquots from the same tube.

Principles of the Procedure

Methyl red-positive organisms produce high levels of acid during fermentation of dextrose, overcome the phosphate buffer system and produce a red color upon the addition of the methyl red pH indicator.

In the Voges-Proskauer test, the red color produced by the addition of potassium hydroxide to cultures of certain microbial species is due to the ability of the organisms to produce a neutral end product, acetoin (acetylmethylcarbinol), from the fermentation of dextrose.[3] The acetoin is oxidized in the presence of oxygen and alkali to produce a red color.[3] This is a positive Voges-Proskauer reaction.

Formulae

Difco™ MR-VP Medium

Approximate Formula* Per Liter

Buffered Peptone	7.0	g
Dipotassium Phosphate	5.0	g
Dextrose	5.0	g

BBL™ MR-VP Broth

Approximate Formula* Per Liter

Pancreatic Digest of Casein	3.5	g
Peptic Digest of Animal Tissue	3.5	g
Potassium Phosphate	5.0	g
Dextrose	5.0	g

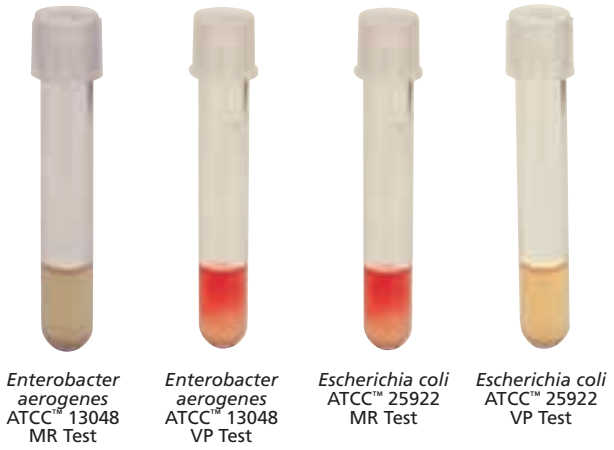
**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 17 g of the powder in 1 L of purified water. Mix thoroughly.
2. If necessary, heat slightly to dissolve.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Using a light inoculum, inoculate tubes of MR-VP media with 18- to 24-hour pure cultures. Incubate tubes aerobically at 35 ± 2°C for a minimum of 48 hours but preferably for 5 days.



Prepare the methyl red indicator by dissolving 0.1 g of methyl red in 300 mL of 95% ethyl alcohol. Add sufficient purified water to make 500 mL.

After the appropriate incubation period, aseptically remove aliquots (1 mL for the VP test) of the medium and conduct the following tests:

1. Methyl Red Test – Add 5 drops of methyl red indicator to an aliquot of the broth. Interpret the color result immediately.
2. Voges-Proskauer Test – Empty the contents (15 drops) from the reagent A dropper and 5 drops from the reagent B dropper into 1 mL of broth culture. Shake well after the addition of each reagent to aerate the sample.

Expected Results

1. Methyl Red Test
   a. Positive – red color at surface of the medium.
   b. Negative – yellow color at surface of the medium.
2. Voges-Proskauer Test
   A positive reaction is indicated by the development of a distinct red color which occurs within 5 minutes.

Certain species within *Enterobacteriaceae* genera may react differently or give variable results. Consult appropriate texts for reactions of specific species.[3-6]

Limitations of the Procedure

1. Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within the *Enterobacteriaceae*.
2. A precipitate may form in the potassium hydroxide reagent solution. This precipitate has not been shown to reduce the effectiveness of the reagent.
3. Most members of the family *Enterobacteriaceae* give either a positive MR test or a positive VP test. However, certain organisms such as *Hafnia alvei* and *Proteus mirabilis* may give a positive result for both tests.
4. Incubation time for the Methyl Red test cannot be shortened by increasing the dextrose concentration in the medium or by heavily inoculating the broth.[7]
5. Incubate MR-negative tests for more than 48 hours and test again.
6. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.[7]
7. VP reagents must be added in the order and the amounts specified or a weak-positive or false-negative reaction may occur. A weak-positive reaction may be masked by a copper-like color which may form due to the reaction of KOH and α-naphthol.[7]
8. Read the VP test within 1 hour of adding the reagents. The KOH and α-naphthol may react to form a copper-like color, causing a potential false-positive interpretation.[7]
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials,[8] the use of media low in these substances (such as MR-VP media) is recommended for this test.

References

1. Voges and Proskauer. 1898. Z. Hyg. *28*:20.
2. Clark and Lubs. 1915. J. Infect. Dis. *17*:160.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore, Md.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
7. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Barritt. 1936. J. Pathol. *42*:441.

Availability

Difco™ MR-VP Medium

AOAC BAM CCAM COMPF ISO SMD SMWW USDA

Cat. No. 216300 Dehydrated – 500 g

BBL™ MR-VP Broth

AOAC BAM COMPF ISO SMD SMWW USDA

Cat. No. 211383 Dehydrated – 500 g
221667 Prepared Tubes – Pkg. of 10
221668 Prepared Tubes – Ctn. of 100

Difco™/BBL™ Voges-Proskauer Reagent A

Cat. No. 261192 Droppers – Ctn. of 50

Difco™/BBL™ Voges-Proskauer Reagent B

Cat. No. 261193 Droppers – Ctn. of 50

MYP Agar Antimicrobic Vial P

Intended Use

MYP Agar is used with Egg Yolk Enrichment 50% and Antimicrobic Vial P for enumerating *Bacillus cereus* from foods.

Summary and Explanation

Mossel et al.[1] formulated Mannitol-Egg Yolk-Polymyxin (MYP) Agar to isolate and enumerate *Bacillus cereus* from foods. This medium differentiates *B. cereus* from other bacteria based on its resistance to polymyxin, lack of mannitol fermentation and presence of lecithinase.[2,3] *B. cereus* is commonly found in nature, on vegetables and in some processed foods.[4] Under favorable circumstances the microorganism grows to sufficient numbers and causes gastrointestinal illness.[4] Outbreaks of foodborne illness have been associated with boiled and cooked rice, cooked meats and cooked vegetables.[5]

MYP Agar is a recommended medium for testing foods.[4-6]

Principles of the Procedure

MYP Agar contains beef extract and peptone as sources of carbon, nitrogen, vitamins and minerals. D-Mannitol is the carbohydrate source. Phenol red is the pH indicator. Agar is the solidifying agent. Egg Yolk Enrichment 50% provides lecithin. Antimicrobic Vial P is polymyxin B which inhibits the growth of most other bacteria.

Bacteria that ferment mannitol produce acid products and form colonies that are yellow. Bacteria that produce lecithinase hydrolyze the lecithin and a zone of white precipitate forms around the colonies. *B. cereus* is typically mannitol-negative (pink-red colonies) and lecithinase-positive (zone of precipitate around the colonies).

User Quality Control

Identity Specifications

Difco™ MYP Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	46 g soluble in 900 mL purified water upon boiling. Solution is red, slightly opalescent.
Prepared Appearance:	Red, very slightly to slightly opalescent without significant precipitate.
Reaction of 46 g/900 mL at 25°C:	pH 7.2 ± 0.1

Difco™ Antimicrobic Vial P

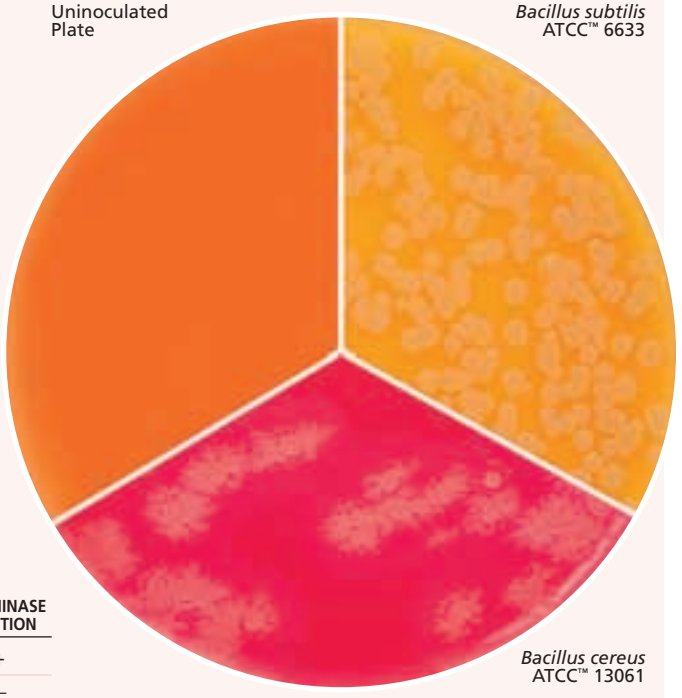
Dehydrated Appearance:	White cake or powder.

Cultural Response

Difco™ MYP Agar

Prepare the medium per label directions. Supplement with Egg Yolk Enrichment 50% and Antimicrobic Vial P. Inoculate and incubate at 30 ± 2°C for 18-48 hours. Lecithinase reaction is read as a zone of precipitate. Colonies that ferment mannitol are yellow.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	MANNITOL FERMENTATION	LECITHINASE REACTION
Bacillus cereus	13061	30-300	Good	–	+
Bacillus subtilis	6633	30-300	Good	+	–
Pseudomonas aeruginosa	27853	10^3-2×10^3	Inhibition	–	–



Formulae

Difco™ MYP Agar

Approximate Formula* Per 900 mL

Beef Extract	1.0	g
Peptone	10.0	g
D-Mannitol	10.0	g
Sodium Chloride	10.0	g
Phenol Red	25.0	mg
Agar	15.0	g

Difco™ Antimicrobic Vial P

Approximately 30,000 units polymyxin B per vial.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ MYP Agar

1. Suspend 46 g of the powder in 900 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense 225 mL into 500 mL flasks.
4. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
5. Aseptically add 12.5 mL Egg Yolk Enrichment 50% and 4.1 mL Antimicrobic Vial P rehydrated with 5 mL sterile water (25,000 units of polymyxin B). Mix thoroughly.
6. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Antimicrobic Vial P (Polymyxin B)

1. To rehydrate, aseptically add 5 mL sterile purified water (to achieve the desired concentration for MYP Agar).
2. Rotate in an end-over-end motion to dissolve the contents completely.

Procedure

Consult appropriate references.[4-6]

Expected Results

Consult appropriate references.[4-6]

References

1. Mossel, Koopman and Jongerius. 1967. Appl. Microbiol. *15*:650.
2. Donovan. 1958. J. Appl. Bacteriol. *21*:100.
3. Coliner. 1948. J. Bacteriol. *55*:777.
4. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
5. Bennett and Belay. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ MYP Agar

AOAC BAM COMPF ISO USDA

Cat. No. 281010 Dehydrated – 500 g

Europe

Cat. No. 257004 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251264 Prepared Plates – Pkg. of 20*

Difco™ Antimicrobic Vial P

AOAC BAM COMPF ISO USDA

Cat. No. 232681 Vial – 6 × 10 mL*

Difco™ Egg Yolk Enrichment 50%

AOAC BAM COMPF ISO USDA

Cat. No. 233471 Tube – 12 × 10 mL*
233472 Bottle – 6 × 100 mL*

**Store at 2-8°C.*

MacConkey Agars
MacConkey Agar • MacConkey Agar Base
MacConkey Agar without Crystal Violet
MacConkey Agar without Crystal Violet or Salt
MacConkey Agar without Salt

Intended Use

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical,[1-3] dairy,[4] food,[5-7] water,[8] pharmaceutical,[9-11] cosmetic,[6,7] and other industrial sources.

MacConkey Agar is used for isolating and differentiating lactose-fermenting from lactose-nonfermenting gram-negative enteric bacilli.

MacConkey Agar Base is used with added carbohydrate in differentiating coliforms based on fermentation reactions.

MacConkey Agar without Crystal Violet is used for isolating and differentiating enteric microorganisms while permitting growth of staphylococci and enterococci. The medium can be used also to separate *Mycobacterium fortuitum* and *M. chelonae* from other rapidly growing mycobacteria.

MacConkey Agar without Crystal Violet or Salt and MacConkey Agar without Salt are used for isolating and differentiating gram-negative bacilli while suppressing the swarming of most *Proteus* species.

MacConkey Agar meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[9-11] performance specifications, where applicable.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ MacConkey Agar

Dehydrated Appearance: Pink to pinkish beige, free-flowing, homogeneous.

Solution: 5.0% solution, soluble in purified water upon boiling. Solution is reddish-purple, slightly opalescent.

Prepared Appearance: Pinkish red, slightly opalescent.

Reaction of 5.0% Solution at 25°C: pH 7.1 ± 0.2

Difco™ MacConkey Agar Base

Dehydrated Appearance: Pinkish beige, free-flowing, homogeneous.

Solution: 4.0% solution, soluble in purified water upon boiling. Solution is red, very slightly to slightly opalescent.

Prepared Appearance: Red, slightly opalescent.

Reaction of 4.0% Solution at 25°C: pH 7.1 ± 0.2

Difco™ MacConkey Agar without Crystal Violet

Dehydrated Appearance: Pinkish beige, free-flowing, homogeneous.

Solution: 5.2% solution, soluble in purified water upon boiling. Solution is reddish orange, clear to very slightly opalescent.

Prepared Appearance: Reddish orange, clear to very slightly opalescent.

Reaction of 5.2% Solution at 25°C: pH 7.4 ± 0.2

Difco™ MacConkey Agar without Salt

Dehydrated Appearance: Beige to pinkish beige, free-flowing, homogeneous.

Solution: 4.7% solution, soluble in purified water upon boiling. Solution is reddish orange, slightly opalescent.

Prepared Appearance: Reddish orange, slightly opalescent.

Reaction of 4.7% Solution at 25°C: pH 7.4 ± 0.2

Cultural Response

Difco™ MacConkey Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (incubate *E. coli* ATCC 25922 for 40-48 hours). For *E. coli* ATCC 8739, inoculate in duplicate and incubate one plate at 30-35°C for 18-24 hours and the other plate at 35-37°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–	–
Escherichia coli	25922	30-300	Good	Pink to red	+
Proteus mirabilis	12453	30-300	Good	Colorless	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless	–
Escherichia coli	8739	<100	Growth (18-24 hours at 30-35°C)	Pink to red	+
Escherichia coli	8739	<100	Growth (18-72 hours at 35-37°C)	Pink to red	+

Difco™ MacConkey Agar Base

Prepare the medium per label directions without and with 1% added lactose. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR PLAIN	COLONY COLOR W/LACTOSE	BILE PPT.
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–	–	–
Escherichia coli	25922	30-300	Good	Colorless	Pink to red	+ (w/lactose)
Proteus mirabilis	12453	30-300	Good	Colorless	Colorless	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless	Colorless	–

Difco™ MacConkey Agar without Crystal Violet

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	30-300	Good	Red	–
Escherichia coli	25922	30-300	Good	Pink to red	–
Proteus mirabilis	12453	30-300	Good	Colorless	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless	–
Staphylococcus aureus	25923	30-300	Good	Pink to red	–

Difco™ MacConkey Agar without Salt

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	33186	30-300	Good	Red	–
Escherichia coli	25922	30-300	Good	Pink to red	–
Proteus mirabilis	12453	30-300	Good	Colorless, no swarming	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless	–
Shigella flexneri	12022	30-300	Good	Colorless	–

Continued

Identity Specifications

BBL™ MacConkey Agar

Dehydrated Appearance: Fine, homogenous, may contain dark particles.

Solution: 5.0% solution, soluble in purified water upon boing. Solution is medium to dark, rose to brown-rose with or without a trace orange tint; clear to slightly hazy.

Prepared Appearance: Medium to dark, rose to brown-rose with or without a trace orange tint; clear to slightly hazy.

Reaction of 5.0% Solution at 25°C: pH 7.1 ± 0.2

BBL™ MacConkey Agar (prepared)

Appearance: Medium-dark, rose-tan and trace hazy.

Reaction at 25°C: pH 7.1 ± 0.2

BBL™ MacConkey Agar without Crystal Violet

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 5.2% solution, soluble in purified water upon boiling. Solution is medium, red-orange to red-rose, slightly hazy to hazy.

Prepared Appearance: Medium, red-orange to red-rose, slightly hazy to hazy.

Reaction of 5.2% Solution at 25°C: pH 7.4 ± 0.2

BBL™ MacConkey Agar without Crystal Violet or Salt

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 4.37% solution, soluble in purified water upon boiling. Solution is medium, red-orange to red-rose, slightly hazy to hazy.

Prepared Appearance: Medium, red-orange to red-rose, slightly hazy to hazy.

Reaction of 4.37% Solution at 25°C: pH 7.4 ± 0.2

Cultural Response

BBL™ MacConkey Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours. For *E. coli* ATCC 8739, inoculate in duplicate and incubate one plate at 30-35°C for 18-24 hours and the other plate at 35-37°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^4-10^5	Partial to complete inhibition	–	–
Escherichia coli	25922	10^3-10^4	Good	Red to rose-red	+
Proteus mirabilis	12453	10^3-10^4	Good	Colorless	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–
Shigella flexneri	12022	10^3-10^4	Good	Colorless	–
Escherichia coli	8739	<100	Growth (18-24 hours at 30-35°C)	Red to rose-red	+
Escherichia coli	8739	<100	Growth (18-72 hours at 35-37°C)	Red to rose-red	+

BBL™ MacConkey I Agar (prepared)

Inoculate and incubate at 35 ± 2°C for 18-24 hours. Incubate *E. coli* ATCC 8739 at 30-35°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^4-10^5	Partial to complete inhibition	–	–
Escherichia coli	25922	10^3-10^4	Good	Red to rose-red	+
Pseudomonas aeruginosa	10145	10^3-10^4	Good	Greenish yellow	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	No reaction	–
Shigella dysenteriae	9361	10^3-10^4	Good	No reaction	–
Escherichia coli	8739	10-100	Growth	Red to rose-red	+

BBL™ MacConkey Agar without Crystal Violet

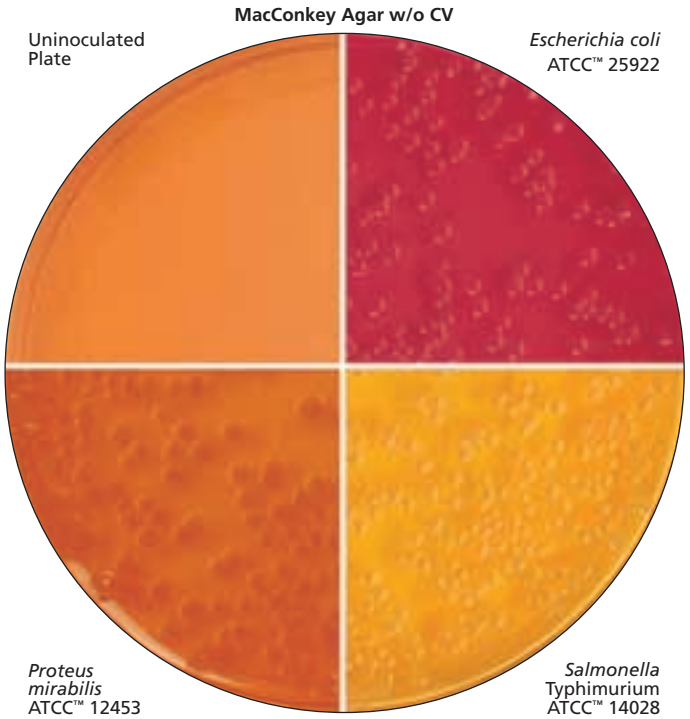
Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours and up to 48 hours if necessary (up to 11 days for *M. fortuitum*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-10^4	Good	Rose red	–
Escherichia coli	25922	10^3-10^4	Good	Pink to rose red	–
Mycobacterium fortuitum	6841	10^3-10^4	Good	Rose red	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–
Staphylococcus aureus	25923	10^3-10^4	Good	Pink to rose red	–

BBL™ MacConkey Agar without Crystal Violet or Salt

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours and up to 48 hours if necessary (up to 11 days for *M. fortuitum*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-10^4	Good	Rose red	–
Escherichia coli	25922	10^3-10^4	Good	Pink to rose red	–
Proteus mirabilis	12453	10^3-10^4	Good	Colorless, no swarming	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–



Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.[12]

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved the growth of *Shigella* and *Salmonella* strains. These modifications included the addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations. The formula improvements gave improved differential reactions between these enteric pathogens and the coliform group.

MacConkey Agar contains crystal violet and bile salts that inhibit gram-positive organisms and allow gram-negative organisms to grow. Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation. Colonies that do not ferment lactose (such as typhoid, paratyphoid and dysentery bacilli) remain colorless. When lactose nonfermenters grow in proximity to coliform colonies, the surrounding medium appears as cleared areas. MacConkey Agar is listed as one of the recommended media for the isolation of *E. coli* from nonsterile pharmaceutical products.[9]

MacConkey Agar Base is prepared without added carbohydrates, which permits their addition either individually or in combination. It is recommended that carbohydrates such as sucrose or lactose be added in a concentration of 1% to the basal medium.

MacConkey Agar without Crystal Violet is a differential medium that is less selective than MacConkey Agar. The lack of crystal violet permits the growth of *Staphylococcus* and *Enterococcus*. Staphylococci produce pale pink to red colonies and enterococci produce compact tiny red colonies either on or beneath the surface of the medium. The medium is used also to separate *Mycobacterium fortuitum* and *M. chelonae* from other rapidly growing mycobacteria.[13,14]

MacConkey Agar without Crystal Violet or Salt and MacConkey Agar without Salt (which also lacks crystal violet) are differential media used for isolating and cultivating gram-negative enteric organisms and gram-positive cocci from waters, feces and other sources suspected of containing these organisms, as well as limiting the swarming of *Proteus* species.

Principles of the Procedure

Peptones are sources of nitrogen and other nutrients. Yeast extract is a source of trace elements, vitamins, amino acids and carbon. Lactose is a fermentable carbohydrate. When lactose is fermented, a local pH drop around the colony causes a color change in the pH indicator (neutral red) and bile precipitation. Bile salts, bile salts no. 3, oxgall and crystal violet are selective agents that inhibit growth of gram-positive organisms. Sodium chloride maintains osmotic balance in the medium. Magnesium sulfate is a source of divalent cations. Agar is the solidifying agent.

Formulae

Difco™ MacConkey Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	17.0	g
Peptones (meat and casein)	3.0	g
Lactose	10.0	g
Bile Salts No. 3	1.5	g
Sodium Chloride	5.0	g
Agar	13.5	g
Neutral Red	0.03	g
Crystal Violet	1.0	mg

Difco™ MacConkey Agar Base

Consists of the same ingredients without the lactose.

BBL™ MacConkey Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	17.0	g
Peptones (meat and casein)	3.0	g
Lactose	10.0	g
Bile Salts	1.5	g
Sodium Chloride	5.0	g
Agar	13.5	g
Neutral Red	0.03	g
Crystal Violet	1.0	mg

Difco™ MacConkey Agar without Crystal Violet

Approximate Formula* Per Liter

Peptone	20.0	g
Lactose	10.0	g
Bile Salts	5.0	g
Sodium Chloride	5.0	g
Agar	12.0	g
Neutral Red	0.05	g

BBL™ MacConkey Agar without Crystal Violet

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Lactose	10.0	g
Bile Salts	5.0	g
Sodium Chloride	5.0	g
Agar	12.0	g
Neutral Red	0.05	g

Difco™ MacConkey Agar without Salt

Approximate Formula* Per Liter		
Peptone	20.0	g
Lactose	10.0	g
Bile Salts	5.0	g
Agar	12.0	g
Neutral Red	75.0	mg

BBL™ MacConkey Agar without Crystal Violet or Salt

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	10.0	g
Yeast Extract	10.0	g
Lactose	10.0	g
Oxgall	5.0	g
Magnesium Sulfate	0.2	g
Agar	12.0	g
Neutral Red	75.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ MacConkey Agar – 50 g;
   BBL™ MacConkey Agar – 50 g;
   Difco™ MacConkey Agar Base – 40 g;
   Difco™ MacConkey Agar without Crystal Violet – 52 g;
   BBL™ MacConkey Agar without Crystal Violet – 52 g;
   BBL™ MacConkey Agar without Crystal Violet or Salt – 47.3 g;
   Difco™ MacConkey Agar without Salt – 47 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.

NOTE: If MacConkey Agar Base is to be used within 12 hours, omit autoclaving and gently boil medium for 5 minutes. Add 1% carbohydrate before or after autoclaving, depending upon heat lability. The surface of MacConkey agars without salt should be thoroughly air-dried prior to inoculation.

4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[1-3]

For food or dairy samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[4-7]

For cosmetics, water, or other industrial samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[6-11]

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *E. coli* using MacConkey Agar.[9]

Procedure

Refer to appropriate standard references for details on test methods to obtain isolated colonies from specimens or samples using MacConkey Agar.[1-11] Incubate plates for 18-72 hours at 35 ± 2°C under appropriate atmospheric conditions, or as instructed in the standard reference.[1-11]

Expected Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or without a zone of precipitated bile. Lactose-nonfermenting organisms grow as colorless or clear colonies.

Swarming by *Proteus* spp. is reduced on MacConkey agars without salt.

On MacConkey Agar without Crystal Violet and MacConkey agars without salt, staphylococci produce pale pink to red colonies and enterococci produce tiny red colonies; these organisms are inhibited on MacConkey Agar.

On MacConkey Agar without Crystal Violet, potentially pathogenic rapid growers of the *M. fortuitum* complex usually grow in 5-11 days, while the commonly saprophytic species are inhibited.[3,13]

On MacConkey agars without salt, the swarming of *Proteus* is reduced.

Limitations of the Procedure

1. Although MacConkey media are selective primarily for gram-negative enteric bacilli, biochemical and, if indicated, serological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[1,3]
2. Incubation of MacConkey Agar plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.[14]
3. Some strains of *M. smegmatis* from humans may grow on MacConkey Agar without Crystal Violet, but these strains can be differentiated from *M. fortuitum* complex by the 3-day arylsulfatase test.[9]

References

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Availability

Difco™ MacConkey Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP JP MCM9 SMD SMWW USP

Cat. No.	212123	Dehydrated – 500 g†
	212122	Dehydrated – 2 kg†
	275300	Dehydrated – 10 kg†

BBL™ MacConkey Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP JP MCM9 SMD SMWW USP

Cat. No.	211387	Dehydrated – 500 g†
	211390	Dehydrated – 5 lb (2.3 kg)†
	211391	Dehydrated – 25 lb (11.3 kg)†

BBL™ MacConkey I Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP JP MCM9 SMD SMWW USP

United States and Canada

Cat. No.	215197	Prepared Plates – Pkg. of 20*†
	297064	Prepared Plates – Ctn. of 100*†

Difco™ MacConkey Agar Base

Cat. No.	281810	Dehydrated – 500 g

Difco™ MacConkey Agar without Crystal Violet

Cat. No.	247010	Dehydrated – 500 g

BBL™ MacConkey Agar without Crystal Violet

Cat. No.	211393	Dehydrated – 500 g

Europe

Cat. No.	256008	Prepared Plates – Pkg. of 20*

BBL™ MacConkey Agar without Crystal Violet or Salt

Cat. No.	294584	Dehydrated – 500 g

Difco™ MacConkey Agar without Salt

Cat. No.	233120	Dehydrated – 500 g
	233110	Dehydrated – 10 kg

Europe

Cat. No.	256009	Prepared Plates – Pkg. of 20*
	257286	Prepared Plates – Ctn. of 120*

** Store at 2-8°C.*
† QC testing performed according to USP/EP/JP performance specifications.

MacConkey II Agar • MacConkey II Agar with MUG

Intended Use

MacConkey II Agar is a slightly selective and differential medium for the detection of coliform organisms and enteric pathogens.

MacConkey II Agar with MUG is used for the presumptive identification of *Escherichia coli*.

MacConkey II Agar meets *United States Pharmacopeia* (*USP*) performance specifications.

Summary and Explanation

The **BBL**™ MacConkey II Agar formulation was made available in 1983. It was specially designed to improve the inhibition of swarming *Proteus* species, to achieve more definitive differentiation of lactose fermenters and nonfermenters, and for the promotion of superior growth of enteric pathogens.

Trepeta and Edberg[1] modified MacConkey Agar by the incorporation of MUG (4-methylumbelliferyl-β-D-glucuronide). The resulting medium allowed the authors to presumptively identify *E. coli* from the primary plating medium within 5 minutes.

Principles of the Procedure

MacConkey II Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci.

Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Most strains (96-97%) of *E. coli* produce β-D-glucuronidase.[2] The enzyme hydrolyzes MUG to yield 4-methylumbelliferone, a compound that fluoresces under long-wave (366 nm) UV light. The addition of MUG to the formulation allows β-D-glucuronidase-positive strains of *E. coli* to fluoresce blue-green when examined under UV light.

BBL MacConkey II Agar with MUG contains 0.1 g of MUG per liter of MacConkey II Agar.

Formula

BBL™ MacConkey II Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	17.0	g
Pancreatic Digest of Casein	1.5	g
Peptic Digest of Animal Tissue	1.5	g
Lactose	10.0	g
Bile Salts	1.5	g
Sodium Chloride	5.0	g
Agar	13.5	g
Neutral Red	0.03	g
Crystal Violet	1.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

BBL™ MacConkey II Agar

Dehydrated Appearance:	Fine, homogeneous, may contain dark particles.
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is medium to dark, rose to brown-rose, clear to slightly hazy.
Prepared Appearance:	Medium to dark, rose to brown-rose, clear to slightly hazy.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

BBL™ MacConkey II Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^4-10^5	Partial to complete inhibition	–	–
Escherichia coli	25922	10^3-10^4	Good	Pink to red	+
Proteus mirabilis	12453	10^3-10^4	Good, inhibition of swarming	Colorless	–
Pseudomonas aeruginosa	10145	10^3-10^4	Good	Colorless to blue to green to pink	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–

Directions for Preparation from Dehydrated Product

1. Suspend 50 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Incubate plates, protected from light, at 35 ± 2°C (do not use CO_2-enriched atmosphere with MacConkey II Agar) or other appropriate temperature for 18-24 hours; if negative after 24 hours on MUG-containing medium, reincubate an additional 24 hours.

Expected Results

After incubation, examine the medium macroscopically for typical colonies. Colonies of lactose-fermenting bacteria appear pink to rose-red in color and may be surrounded by a zone of bile precipitation, while lactose-nonfermenting colonies are colorless.

Examine MacConkey II Agar with MUG under long-wavelength UV light (366 nm). β-D-glucuronidase-positive colonies have a blue-green fluorescence; β-D-glucuronidase-negative colonies do not fluoresce.

Limitations of the Procedure

Not all strains of *E. coli* ferment lactose or produce β-D-glucuronidase. Some strains of *Salmonella* and *Shigella* produce β-D-glucuronidase and will fluoresce.[3] A small percentage of *Yersinia* and streptococci have been reported to fluoresce.[4] Additional biochemical or serological tests are necessary for definitive identification.

References

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2. Killian and Bulow. 1976. Acta Pathol. Microbiol. Scand. Sec. B. *84*:245.
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Availability

BBL™ MacConkey II Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP MCM9 SMD SMWW USP

Cat. No. 212306 Dehydrated – 500 g
292861 Dehydrated – 10 kg

United States and Canada
Cat. No. 221172 Prepared Plates – Pkg. of 20*
221270 Prepared Plates – Ctn. of 100*

Europe
Cat. No. 254025 Prepared Plates – Pkg. of 20*
254078 Prepared Plates – Ctn. of 120*

Japan
Cat. No. 251172 Prepared Plates – Pkg. of 20*
251270 Prepared Plates – Ctn. of 100*
251577 Prepared Plates (Deep Fill) – Ctn. of 100*

BBL™ MacConkey II Agar//Columbia CNA Agar with 5% Sheep Blood

BS12 CMPH2 MCM9

Cat. No. 221600 Prepared **I Plate**™ Dishes – Pkg. of 20*
221601 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ MacConkey II Agar//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

BS12 CMPH2 MCM9

United States and Canada
Cat. No. 221290 Prepared **I Plate**™ Dishes – Pkg. of 20*
221291 Prepared **I Plate**™ Dishes – Ctn. of 100*

Europe
Cat. No. 251290 Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ MacConkey II Agar//Levine EMB Agar

Cat. No. 295969 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ MacConkey II Agar//Columbia PNA Agar with 5% Sheep Blood

Cat. No. 297272 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ MacConkey II Agar//Chocolate II Agar//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No. 299580 Prepared **Y Plate**™ Dishes – Ctn. of 100*

BBL™ MacConkey II Agar with MUG

Cat. No. 221938 Prepared Plates – Pkg. of 20*

BBL™ MacConkey II Agar with MUG//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No. 221949 Prepared **I Plate**™ Dishes – Pkg. of 20*

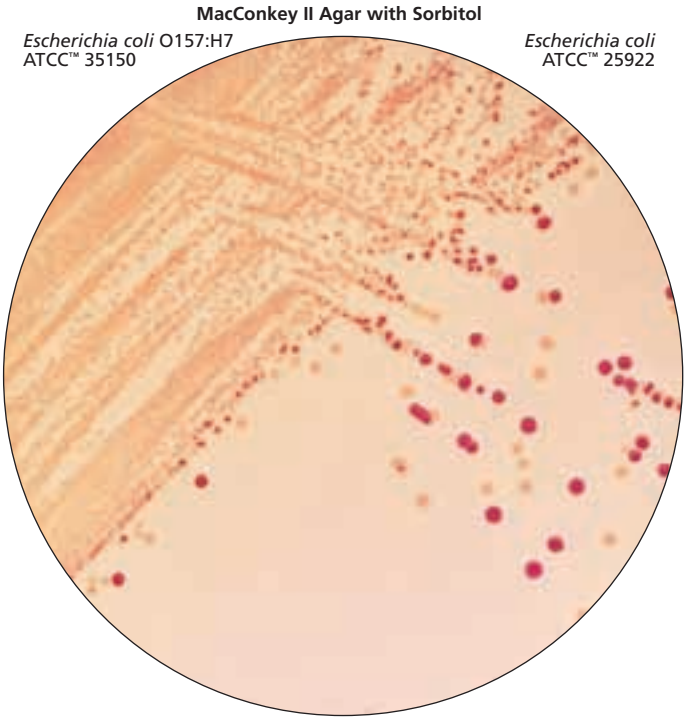
**Store at 2-8°C.*

MacConkey Agars with Sorbitol
MacConkey Sorbitol Agar • MacConkey II Agar with Sorbitol • Sorbitol MacConkey II Agar with Cefixime and Tellurite

Intended Use

MacConkey Sorbitol Agar and MacConkey II Agar with Sorbitol are selective and differential media for the detection of sorbitol-nonfermenting *Escherichia coli* serotype O157:H7 associated with hemorrhagic colitis. These media are also referred to as "Sorbitol MacConkey Agar."

Sorbitol MacConkey II Agar with Cefixime and Tellurite (SMAC-CT) is a more selective and differential medium designed to inhibit *Proteus mirabilis*, non-O157 *E. coli* strains and other sorbitol-nonfermenting strains.



Summary and Explanation

Escherichia coli serotype O157:H7 is a human pathogen associated with hemorrhagic colitis that results from the action of a shiga-like toxin (SLT).[1] On standard MacConkey Agar containing lactose, this strain is indistinguishable from other lactose-fermenting *E. coli*. Unlike most *E. coli* strains, *E. coli* O157:H7 ferments sorbitol slowly or not at all. Therefore, the efficacy of MacConkey Agar containing sorbitol instead of lactose as a differential medium for the detection of *E. coli* O157:H7 in stool cultures was determined. Field trial results showed that the growth of *E. coli* O157:H7 on MacConkey Agar with Sorbitol was heavy and occurred in almost pure culture as colorless sorbitol-nonfermenting colonies. Most organisms

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ MacConkey Sorbitol Agar

Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous (may contain dark particles).
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is reddish-purple, very slightly to slightly opalescent.
Prepared Appearance:	Reddish-purple, slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Difco™ MacConkey Sorbitol Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-2×10^3	Marked to complete inhibition	–	–
Escherichia coli	25922	10^2-10^3	Good	Pink-red	+
Escherichia coli O157:H7	35150	10^2-10^3	Good	Colorless	–

Identity Specifications

BBL™ MacConkey II Agar with Sorbitol

Dehydrated Appearance:	Fine, homogeneous, may contain dark particles.
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is medium to dark, rose to brown rose, with or without a trace orange tint, clear to slightly hazy.
Prepared Appearance:	Medium to dark, rose to brown-rose, with or without a trace orange tint, clear to slightly hazy.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

BBL™ MacConkey II Agar with Sorbitol

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (42-48 hours for *E. coli* ATCC™ 25922).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Escherichia coli	25922	10^3-10^4	Good	Pink to red to rose-red	+
Escherichia coli O157:H7	35150	10^3-10^4	Good	Colorless	–
Proteus mirabilis	12453	10^3-10^4	Good	Colorless	–

of the fecal flora ferment sorbitol and appear pink on this medium. MacConkey Agar with Sorbitol, therefore, permits ready recognition of *E. coli* O157:H7 in stool cultures.[1-3]

The addition of cefixime and tellurite significantly reduces the number of sorbitol nonfermenters that need to be screened during the attempted isolation of *E. coli* O157:H7.[4,5]

Principles of the Procedure

MacConkey Sorbitol Agar and MacConkey II Agar with Sorbitol, modified MacConkey agars using sorbitol instead of lactose, are only slightly selective, since the concentration of bile salts, which inhibits gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci. MacConkey II Agar with Sorbitol is also formulated to reduce swarming of *Proteus* species.

Differentiation of enteric microorganisms is achieved by the combination of sorbitol and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate sorbitol.

SMAC-CT is modified MacConkey II Agar using sorbitol instead of lactose and with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) added. Cefixime inhibits *Proteus* spp. and tellurite inhibits non-O157 *E. coli* and other organisms, thus improving the selectivity of SMAC-CT for *E. coli* O157:H7.

Formulae

Difco™ MacConkey Sorbitol Agar

Approximate Formula* Per Liter

Peptone	15.5	g
Proteose Peptone	3.0	g
D-Sorbitol	10.0	g
Bile Salts	1.5	g
Sodium Chloride	5.0	g
Agar	15.0	g
Neutral Red	0.03	g
Crystal Violet	1.0	mg

BBL™ MacConkey II Agar with Sorbitol

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	17.0	g
Pancreatic Digest of Casein	1.5	g
Peptic Digest of Animal Tissue	1.5	g
D-Sorbitol	10.0	g
Bile Salts	1.5	g
Sodium Chloride	5.0	g
Agar	13.5	g
Neutral Red	0.03	g
Crystal Violet	1.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 50 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Prepare plated medium from tubed agar deeps by liquefying the medium in boiling water, cooling to 45-50°C and pouring into sterile Petri dishes.

Use standard procedures to obtain isolated colonies from specimens. Incubate plates, protected from light, in an inverted position (agar side up) at 35 ± 2°C for 18-24 hours in an aerobic atmosphere without additional CO_2.

Expected Results

After 18-24 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Sorbitol fermenters produce pink to red colonies, some surrounded by zones of precipitated bile, while sorbitol nonfermenters produce colorless colonies.

Most fecal flora will be partially to completely inhibited on SMAC-CT.

Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

Limitations of the Procedure

1. It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO_2-enriched atmosphere.[6]
2. Prolonged incubation of the culture may result in colonies of *E. coli* serotype O157:H7 losing their characteristic colorless appearance. There are additional species of facultatively anaerobic gram-negative rods that do not ferment sorbitol.
3. The color of sorbitol-positive colonies can fade, making them hard to distinguish from sorbitol-negative colonies.[7]

References

1. March and Ratnam. 1986. J. Clin. Microbiol. *23*:869.
2. Centers for Disease Control. 1991. Morbid. Mortal. Weekly Rep. *40*:265.
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5. Sanderson, Gay, Hancock, Gay, Fox and Besser. 1995. J. Clin. Microbiol. *33*:2616.
6. Mazura-Reetz, Neblett and Galperin. 1979. Abstr. C 179, p. 339. Abstr. Annu. Meet. Am. Soc. Microbiol. 1979.
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Availability

Difco™ MacConkey Sorbitol Agar

BAM BS12 CCAM CMPH2 COMPF ISO MCM9 SMWW

Cat. No. 279100 Dehydrated – 500 g

BBL™ MacConkey II Agar with Sorbitol

BAM BS12 CCAM CMPH2 COMPF ISO MCM9 SMWW

Cat. No. 299769 Dehydrated – 500 g

United States and Canada

Cat. No. 297684 Prepared Deeps (Pour Tubes) – Pkg. of 10
297953 Prepared Plates – Pkg. of 10*
298519 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254455 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251147 Prepared Plates – Pkg. of 20*
251767 Prepared Plates – Ctn. of 100*

Mexico

Cat. No. 252637 Prepared Plates – Pkg. of 10*

BBL™ MacConkey II Agar with Sorbitol//SS Agar

Japan

Cat. No. 251163 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ Sorbitol MacConkey II Agar with Cefixime and Tellurite

BAM CCAM ISO SMWW

United States and Canada

Cat. No. 222226 Prepared Plates – Pkg. of 10*
222227 Prepared Plates – Ctn. of 100*

Japan

Cat. No. 251156 Prepared Plates – Pkg. of 20*
251153 Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*

MacConkey Broth

Intended Use

MacConkey Broth is used for the detection of coliform organisms in milk and water.

Meets *United States Pharmacopeia (USP), European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

MacConkey Broth is a modification of the original bile salt broth recommended by MacConkey[4] that contained 0.5% sodium taurocholate and litmus as an indicator. In later publications,[5,6] MacConkey suggested variations of this formulation using neutral red indicator instead of litmus. Childs and Allen[7] demonstrated the inhibitory effect of neutral red and substituted the less inhibitory bromcresol purple. Oxgall in the medium replaces the original sodium taurocholate to inhibit growth of gram-positive organisms.

MacConkey Broth is used for cultivating gram-negative, lactose-fermenting bacilli and as a presumptive test for coliform organisms. It has been used to analyze food,[8] milk[9,10] and water samples[10-13] for coliforms. In addition, this medium has also been used in the rapid detection of shiga-toxin producing *E. coli* in fecal samples.[14] MacConkey Broth is recommended in the *USP* as a test medium for *E. coli* in the microbiological examination of nonsterile products.[1]

Principles of the Procedure

Peptone provides amino acids and other growth factors. Lactose is a carbon energy source for gram-negative lactose-fermenting bacilli. Oxgall inhibits the growth of gram-positive organisms. Bromcresol purple is the indicator.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ MacConkey Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water. Solution is purple, clear.
Prepared Appearance:	Purple, clear.
Reaction of 3.5% Solution at 25°C:	pH 7.3 ± 0.1

BBL™ MacConkey Broth (prepared)

Appearance:	Purple and clear.
Reaction at 25°C:	pH 7.3 ± 0.2

Uninoculated Tube

Escherichia coli ATCC™ 25922

Cultural Response

Difco™ MacConkey Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. For *E. coli* ATCC 8739 and *S. aureus* ATCC 6538, inoculate 100 mL bottles and incubate at 43-44°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID	GAS
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–	–
Escherichia coli	25922	30-300	Good	+	+
Salmonella enterica subsp. *enterica* serotype Choleraesuis var. Kunzendorf	12011	30-300	Good	–	–
Escherichia coli	8739	<100	Growth (at 24 hours)	N/A	N/A
Staphylococcus aureus	6538	>100	No growth (at 48 hours)	N/A	N/A

KEY: + = positive, yellow for acid, gas
– = negative, no change for no acid, no gas

BBL™ MacConkey Broth (prepared)

Inoculate and incubate at 42-44°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID
Escherichia coli	8739	10 - 100	Growth	+
Escherichia coli	25922	10 - 100	Growth	+
Staphylococcus aureus	6538	>100	No growth	–

KEY: + = positive, yellow for acid
– = negative, no change for no acid

Formula

Difco™ MacConkey Broth

Approximate Formula* Per Liter

Oxgall	5.0	g
Pancreatic Digest of Gelatin	20.0	g
Lactose	10.0	g
Bromcresol Purple	0.01	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 35 g of the powder in 1 L of purified water. For testing 10 mL samples, prepare double strength.
2. Dispense in test tubes containing Durham tubes.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For milk or food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[8-10]

For water samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[10-13]

For pharmaceutical samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[1]

Procedure

Refer to appropriate references for details on test methods using MacConkey Broth.[8-13]

Inoculate tubes with the test sample. Incubate tubes at 35 ± 2°C for 18-24 hours in an aerobic atmosphere, or as instructed in appropriate reference.[8-13]

Expected Results

Lactose-fermenting organisms grow very well in MacConkey Broth and produce acid, causing the medium to turn yellow. Gas is also produced, which collects in the Durham tubes. Nonfermenting organisms produce good growth but will not produce acid or gas.

References

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8. Qadri, Buckle and Edwards. 1974. J. Appl. Bact. 37:7-14.
9. Adeleke, Adeniyi and Akinrinmisi. 2000. Afr. J. Biomed. Res. 3:89-92.
10. Hsu and Tsen. 2001. Int. J. Food Microbiol. 64:1-11.
11. World Health Organization. 4 Sept 2008. European standards for drinking water, 2nd ed., online. <www.who.int/water_sanitation_health/dwq/europstand2/en/index.html>.
12. Alivisatos and Papadakis. 1975. J. Appl. Bact. 39:287-293.
13. International Organization for Standardization. 1990. Water quality – Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* – Part 2: Multiple tube (most probable number) method. ISO 9308-2, First ed., 1990-10-01. International Organization for Standardization, Geneva, Switzerland.
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Availability

Difco™ MacConkey Broth

EP ISO JP USP

Cat. No. 220100 Dehydrated – 500 g†

BBL™ MacConkey Broth

EP ISO JP USP

Cat. No. 215177 Prepared Bottles, 100 mL – Pkg. of 10†

Europe

Cat. No. 254957 Prepared Bottles, 100 mL – Ctn. of 25†

† QC testing performed according to USP/EP/JP performance specifications.

Malonate Broth

Intended Use

Malonate Broth is used for differentiating *Enterobacter* from *Escherichia* based on malonate utilization.

Summary and Explanation

Malonate Broth, prepared according to the formula described by Leifson,[1] is a liquid medium containing ammonium sulfate as the only source of nitrogen and malonate as the only source of carbon. Leifson was able to demonstrate that the *Enterobacter* group utilizes malonate whereas the *Escherichia* group is unable to grow on the medium.

Malonate Broth is further described for differentiating *Enterobacteriaceae* in food and dairy products.[2,3] More often, the medium referenced is the modified Edwards and Ewing[4] formulation that contains yeast extract and dextrose. The modification permits growth of organisms that would otherwise fail on the unsupplemented Leifson medium.

User Quality Control

Identity Specifications

Difco™ Malonate Broth

Dehydrated Appearance:	Tan to green, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in purified water. Solution is green, clear.
Prepared Appearance:	Green, clear.
Reaction of 0.8% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Malonate Broth

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	MEDIUM COLOR
Enterobacter aerogenes	13048	Blue
Enterobacter cloacae	13047	Blue
Escherichia coli	25922	Green
Klebsiella pneumoniae	13883	Blue
Salmonella enterica subsp. *arizonae*	13314	Blue
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Green

Uninoculated Tube | *Enterobacter aerogenes* ATCC™ 13048 | *Escherichia coli* ATCC™ 25922

Principles of the Procedure

Malonate Broth contains ammonium sulfate, which is the sole source of nitrogen in the medium; sodium malonate is the sole source of carbon. Dipotassium phosphate and monopotassium phosphate provide buffering capability. Sodium chloride maintains the osmotic balance of the medium. Increased alkalinity resulting from malonate utilization causes the indicator, bromthymol blue, to change color from green to blue.

Formula

Difco™ Malonate Broth

Approximate Formula* Per Liter		
Ammonium Sulfate	2.0	g
Dipotassium Phosphate	0.6	g
Monopotassium Phosphate	0.4	g
Sodium Chloride	2.0	g
Sodium Malonate	3.0	g
Bromthymol Blue	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 8 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Avoid introducing extraneous carbon and nitrogen.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Inoculate tubes with a loopful of test organism.
2. Incubate at 35 ± 2°C for 18-48 hours.
3. Examine tubes for a change in the color of the medium from green to blue.

Expected Results

Malonate utilization is indicated by a change in the color of the medium from green to blue:

Positive: Blue
Negative: Green

Limitation of the Procedure

A slight bluing (blue-green) of the medium may occur after prolonged incubation.[5] In such cases, care should be taken in interpreting results.

References

1. Leifson. 1933. J. Bacteriol. 26:329.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
4. Edwards and Ewing. 1962. *Enterobacteriaceae.* U.S. Public Health Service Bulletin No. 734:19.
5. Oberhofer. 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, N.Y.

Availability

Difco™ Malonate Broth

COMPF SMD

Cat. No. 239520 Dehydrated – 500 g

Malonate Broth, Ewing Modified

Intended Use

Malonate Broth, as modified by Ewing, is used for the differentiation of coliforms and other enteric organisms.

Summary and Explanation

Leifson, in 1933, developed a synthetic liquid medium which differentiated *Aerobacter* (now *Enterobacter*) from *Escherichia* species based on their ability to utilize malonate.[1] The modification, in which dextrose and yeast extract are incorporated, was devised by Ewing et al.[2]

The addition of yeast extract, a source of vitamins, and a relatively small amount of dextrose, a minimal carbon source, is included in Ewing's modification to stimulate the growth of some organisms. The medium, therefore, will support the growth of organisms that cannot utilize malonate or ammonium salt, but any spontaneous alkalinization produced by such organisms is buffered by the phosphate system and counteracted by the acid produced in the fermentation of the small amount of dextrose.[3] An alkaline result (blue color) is only produced in this medium by organisms capable of utilizing malonate and ammonium sulfate.

Principles of the Procedure

An organism that simultaneously can utilize sodium malonate as its carbon source and ammonium sulfate as its nitrogen source produces an alkalinity due to the formation of sodium hydroxide.[3] The alkali changes the color of the bromthymol blue indicator in the medium to light blue to Prussian blue. The color of the medium remains unchanged in the presence of an organism that cannot utilize these substances. Some malonate-negative strains produce a yellow color due to the fermentation of dextrose only, which results in increased acidity causing the pH indicator to change to yellow at a pH of 6.0.

Formula

BBL™ Malonate Broth, Ewing Modified

Approximate Formula* Per Liter		
Yeast Extract	1.0	g
Ammonium Sulfate	2.0	g
Dipotassium Phosphate	0.6	g
Monopotassium Phosphate	0.4	g
Sodium Chloride	2.0	g
Sodium Malonate	3.0	g
Dextrose	0.25	g
Bromthymol Blue	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

BBL™ Malonate Broth, Ewing Modified

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	0.93% solution, soluble in purified water. Solution is light to medium, green, with or without a tint of yellow or blue, clear to slightly hazy.
Prepared Appearance:	Light to medium, green, with or without a tint of yellow or blue, clear to slightly hazy.
Reaction of 0.93% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

BBL™ Malonate Broth, Ewing Modified

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	RECOVERY	MEDIUM COLOR
Enterobacter aerogenes	13048	Good	Blue
Escherichia coli	25922	Good	Yellow-green to gray-green

Directions for Preparation from Dehydrated Product

1. Dissolve 9.3 g of the powder in 1 L of purified water.
2. Dispense and autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate tubes, using a light inoculum, with growth from an 18- to 24-hour pure culture. Incubate tubes with loosened caps for 18-48 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Bacterial genera in which the majority of species yield a positive alkaline reaction (light blue to Prussian blue color throughout the medium) include:

Enterobacter
Klebsiella
Citrobacter

Genera in which the majority of species yield a negative reaction (color of medium is unchanged or yellow) include:

Escherichia
Salmonella
Shigella
Edwardsiella
Yersinia
Serratia
Morganella
Proteus
Providencia

Limitation of the Procedure

Some malonate-positive organisms produce only slight alkalinity. Compare any tube in question with an uninoculated malonate tube. Any trace of blue color after a 48-hour incubation period denotes a positive test. Before making a final negative interpretation, be sure that test tubes have been incubated for 48 hours.[3]

References

1. Leifson. 1933. J. Bacteriol. 26:329.
2. Ewing, Davis and Reavis. 1957. Public Health Lab. *15*:153.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Malonate Broth, Ewing Modified

AOAC BAM COMPF SMD

Cat. No.	211399	Dehydrated – 500 g
	221322	Prepared Tubes – Pkg. of 10

Malt Agar

Intended Use

Malt Agar is used for isolating and cultivating yeasts and molds from food and for cultivating yeast and mold stock cultures.

Summary and Explanation

Malt media for yeasts and molds have been widely used for many years. In 1919, Reddish[1] prepared a satisfactory substitute for beer wort from malt extract. Thom and Church[2] used Reddish's medium for their studies of the aspergilli. Malt Agar was also employed by Fullmer and Grimes[3] for their studies of the growth of yeasts on synthetic media. Malt Agar is included in *Official Methods of Analysis of AOAC International*.[4]

Principles of the Procedure

Malt Agar contains malt extract which provides the carbon, protein and nutrient sources required for the growth of microorganisms. Agar is the solidifying agent. The acidic pH of Malt Agar allows for optimal growth of molds and yeasts while restricting bacterial growth.

Formula

Difco™ and BBL™ Malt Agar

Approximate Formula* Per Liter

Malt Extract	30.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Malt Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, very slightly to slightly opalescent.
Reaction of a 4.5% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

Difco™ Malt Agar

Prepare the medium per label directions. For specific quantities of sterile 1:10 dilution of lactic acid, USP (85%) to add to 100 mL of medium to obtain a pH of 3.5 or 4.5, see the Certificate of Analysis for each lot.* Inoculate and incubate at 30 ± 2°C for 42-48 hours (up to 72 hours if necessary).

**For Certificates of Analysis from Technical Services, phone 800-638-8663 or via the internet at http://regdocs.bd.com/regdocs/searchCOA.do.*

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10^2-10^3	Good
Candida albicans	10231	10^2-10^3	Good
Saccharomyces cerevisiae	9763	10^2-10^3	Good

Identity Specifications

BBL™ Malt Agar

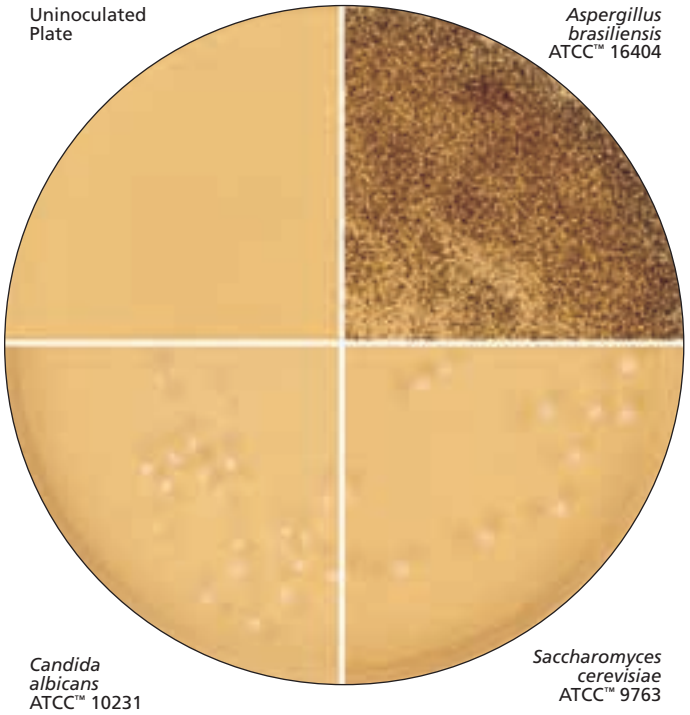
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is medium to dark, yellow to tan, trace hazy to hazy.
Prepared Appearance:	Medium to dark, yellow to tan, trace hazy to hazy.
Reaction of 4.5% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

BBL™ Malt Agar

Prepare the medium per label directions. Inoculate pour plates with *Saccharomyces cerevisiae* and incubate at 25 ± 2°C for 42-48 hours. Inoculate tubes with other test organisms and incubate at 25 ± 2°C for 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	60193	30-300	Good
Saccharomyces cerevisiae	9763	30-300	Good



3. Autoclave at 121°C for 15 minutes. Avoid overheating (and consequent hydrolysis and darkening of the agar with failure to solidify). Note: To lower the pH, add sterile 1:10 lactic acid, USP. DO NOT REHEAT THE MEDIUM.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

Do not heat the medium after addition of acid, as this will hydrolyze the agar and reduce its solidifying properties.

References

1. Reddish. 1919. Abstr. Bacteriol. 3:6.
2. Thom and Church. 1926. The aspergilli. Williams & Wilkins, Baltimore, Md.
3. Fulmer and Grimes. 1923. J. Bacteriol. 8:585.
4. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Malt Agar

AOAC BAM

Cat. No.	224200	Dehydrated – 500 g
	224100	Dehydrated – 10 kg

BBL™ Malt Agar

AOAC BAM

Cat. No.	211401	Dehydrated – 500 g

Bacto™ Malt Extract

Intended Use

Bacto Malt Extract is used for preparing microbiological culture media for the propagation of yeasts and molds.

Summary and Explanation

Bacto Malt Extract is used in the culture of yeasts and molds. This product is very high in carbohydrate content[1] and is suitable for the growth of yeasts and molds because of the high concentration of reduced sugars, especially the maltoses. Malt extract in the agar form is recommended for the detection and isolation of yeasts and molds from dairy products and food. It is also a medium for stock culture maintenance.

Media formulations containing **Bacto** Malt Extract are specified in various standard methods manuals.[2-5]

Principles of the Procedure

Bacto Malt Extract is the water-soluble portion of malted barley. The extraction process breaks down the polysaccharides into simple sugars. After the malting process is complete, the extract is prepared from the malted barley by cracking the grain in a mill and then extracting the grain with a warm liquor. The resulting "wort" is filtered and evaporated or dried under vacuum.[6,7]

User Quality Control

Identity Specifications

Bacto™ Malt Extract

Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in purified water. Solution is medium amber, slightly opalescent to opalescent, with a precipitate.
Reaction of 2.0% Solution at 25°C:	pH 4.5-5.5

Cultural Response

Bacto™ Malt Extract

Prepare a sterile 2% solution of **Bacto** Malt Extract. Inoculate and incubate tubes at 30 ± 2°C for up to 3 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	10231	30-300	Good
Saccharomyces carlsbergensis	9080	30-300	Good

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** Malt Extract in the formula of the medium being prepared. Add product as required.

Procedure

See appropriate references for specific procedures using **Bacto** Malt Extract.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Cote. 1999. *In* Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York, N.Y.
2. Horowitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
6. Bridson and Brecker. 1970. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York, N.Y.
7. How malt is made. Briess Malting Company. 2 Dec. 2002. www.briessmalting.com/hb/hbhow.htm.

Availability

Bacto™ Malt Extract

AOAC BAM COMPF SMWW

Cat. No.	218630	Dehydrated – 500 g
	218610	Dehydrated – 10 kg

Malt Extract Agar • Malt Extract Broth

Intended Use

Malt Extract Agar is used for isolating, cultivating and enumerating yeasts and molds.

Malt Extract Broth is used for cultivating yeasts and molds.

Summary and Explanation

The use of malt and malt extracts for the propagation of yeasts and molds is quite common. Reddish[1] described a culture medium prepared from malt extract that was a satisfactory substitute for wort. Thom and Church,[2] following the formula of Reddish, used malt extract as a base from which they prepared the complete media. Malt Extract Broth is recommended for the examination of yeasts and molds in the U.S. Food and Drug Administration's *Bacteriological Analytical Manual*.[3]

User Quality Control

Identity Specifications

Difco™ Malt Extract Agar

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	3.36% solution, soluble in purified water upon boiling. Solution is very light amber, slightly opalescent.
Prepared Appearance:	Very light amber, slightly opalescent.
Reaction of 3.36% Solution at 25°C:	pH 4.7 ± 0.2

Difco™ Malt Extract Broth

Dehydrated Appearance:	Light beige to beige, free-flowing, homogeneous.
Solution:	1.5% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Very light to light amber, clear.
Reaction of 1.5% Solution at 25°C:	pH 4.7 ± 0.2

Cultural Response

Difco™ Malt Extract Agar or Malt Extract Broth

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours (agar) or 18-72 hours (broth).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10^2-10^3	Good
Candida albicans	10231	10^2-10^3	Good
Saccharomyces cerevisiae	9763	10^2-10^3	Good

Principles of the Procedure

Malt Extract Agar contains maltose as an energy source. Dextrin, a polysaccharide derived from high quality starch, and glycerol are included as carbon sources. Peptone is provided as a nitrogen source. Agar is the solidifying agent.

Malt Extract Broth contains malt extract which provides the carbon, protein, and nutrient sources required for growth of microorganisms. Maltose is added as an energy source. Dextrose is included as a source of fermentable carbohydrate. Yeast extract provides the vitamins and cofactors required for growth and additional sources of nitrogen and carbon.

The acidic pH of Malt Extract Agar and Broth allows for the optimal growth of molds and yeasts while restricting bacterial growth.

Formulae

Difco™ Malt Extract Agar

Approximate Formula* Per Liter

Maltose, Technical	12.75	g
Dextrin	2.75	g
Glycerol	2.35	g
Peptone	0.78	g
Agar	15.0	g

Difco™ Malt Extract Broth

Approximate Formula* Per Liter

Malt Extract	6.0	g
Maltose, Technical	1.8	g
Dextrose	6.0	g
Yeast Extract	1.2	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Malt Extract Agar

1. Suspend 33.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Malt Extract Broth

1. Dissolve 15 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Reddish. 1919. Abstr. Bacteriol. 3:6.
2. Thom and Church. 1926. The aspergilli. Williams & Wilkins, Baltimore, Md.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Malt Extract Agar

BAM COMPF

Cat. No. 211220 Dehydrated – 500 g

Difco™ Malt Extract Broth

BAM

Cat. No. 211320 Dehydrated – 500 g

Mannitol Salt Agar

Intended Use

Mannitol Salt Agar is used for the selective isolation and enumeration of staphylococci from clinical and nonclinical materials.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Koch, in 1942, reported that only staphylococci grow on agar media containing 7.5% sodium chloride.[4] Chapman further studied this phenomenon in greater detail and concluded that the addition of 7.5% sodium chloride to phenol red mannitol agar results in an improved medium for the isolation of plasma-coagulating staphylococci.[5] Mannitol Salt Agar is listed

User Quality Control

Identity Specifications

BBL™ Mannitol Salt Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material and may contain many light to dark red flecks.
Solution:	11.1% solution, soluble in purified water upon boiling. Solution is medium to dark, red to rose; clear to slightly hazy.
Prepared Appearance:	Light to medium rose red, trace orange; clear to hazy.
Reaction of 11.1% Solution at 25°C:	pH 7.4 ± 0.2

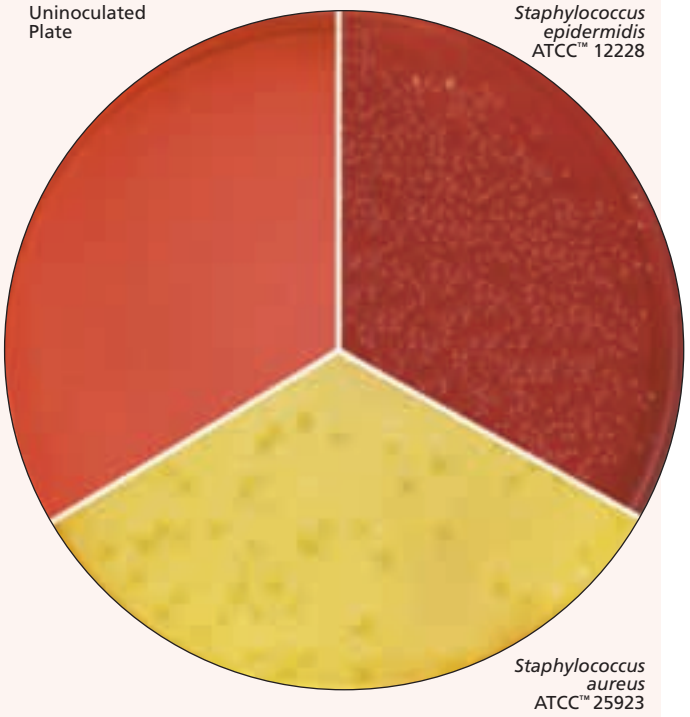
BBL™ Mannitol Salt Agar (prepared)

Appearance:	Light to medium rose red, trace orange; clear to hazy.
Reaction at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Mannitol Salt Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours. Incubate plates with *Staphylococcus aureus* ATCC 6538 and *E. coli* ATCC 8739 at 30-35°C for 18-72 hours.



ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF MEDIUM AROUND COLONY
Proteus mirabilis	12453	$10^4 – 10^5$	Partial to complete inhibition	–
Staphylococcus aureus	25923	$10^3 – 10^4$	Good	Yellow
Staphylococcus epidermidis	12228	$10^3 – 10^4$	Good	Red
Staphylococcus aureus	6538	<100	Growth	N/A
Escherichia coli	8739	>100	No growth	N/A

BBL™ Mannitol Salt Agar (prepared)

Inoculate and incubate at 35 ± 2°C for 48 hours. Incubate plates with *Staphylococcus aureus* ATCC 6538 and *E. coli* ATCC 8739 at 30-35°C for 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF MEDIUM AROUND COLONY
Proteus mirabilis	12453	$10^4 – 10^5$	Partial inhibition	–
Staphylococcus aureus	13150	$10^3 – 10^4$	Good	Yellow
Staphylococcus aureus	25923	$10^3 – 10^4$	Good	Yellow
Staphylococcus epidermidis	12228	$10^3 – 10^4$	Good	Red
Staphylococcus aureus	6538	<100	Growth	N/A
Escherichia coli	8739	>100	No growth	N/A

as one of several media recommended for the enumeration of gram-positive bacteria in cosmetics,[6] clinical specimens,[7-11] and pharmaceutical products.[1] The *USP* General Chapter <62> recommends Mannitol Salt Agar as a test medium for isolating *Staphylococcus aureus* in the Microbiological Examination of Nonsterile Products.[1]

Principles of the Procedure

Mannitol Salt Agar is a nutritive medium due to its content of peptones and beef extract, which supply essential growth factors, such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the differentiation of staphylococcal species. Agar is a solidifying agent.

Formula

BBL™ Mannitol Salt Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Peptic Digest of Animal Tissue	5.0	g
Beef Extract	1.0	g
Sodium Chloride	75.0	g
D-Mannitol	10.0	g
Phenol Red	25.0	mg
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 111 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[7-11]

For cosmetic and pharmaceutical samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[1,13-15]

Procedure

Refer to appropriate standard references for details on test methods to obtain isolated colonies from specimens or samples using Mannitol Salt Agar.[1,6,7,11] Incubate plates at 35 ± 2°C in an aerobic atmosphere for 24-48 hours, or as instructed in the standard reference.[1,6,7,11]

Expected Results

After the recommended incubation period, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Coagulase-positive staphylococci produce growth of yellow colonies with yellow zones. Coagulase negative staphylococci produce small red colonies with no color change to the medium. *Micrococcus* produce large, white to orange colonies, with no color change to the medium. Most other bacteria will be inhibited.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008.The European pharmacopoeia, 6th ed., Supp. 1, 4-1-08, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Koch. 1942. Zentralbl. Bakteriol. Parasitenkd. Abt. I Orig. *149*:122.
5. Chapman. 1945. J. Bacteriol. *50*:201.
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9. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
10. Winn, Koneman, Allen, Janda, Procop, Schreckenberger and Woods (eds.). 2005. Koneman's Color atlas and textbook of diagnostic microbiology, 6th ed. Lippincott Williams & Wilkins, Baltimore, Md.
11. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Mannitol Salt Agar

BAM BS12 CMPH2 EP JP MCM9 USP

Cat. No.	211407	Dehydrated – 500 g†
	211410	Dehydrated – 5 lb (2.3 kg)†
	293689	Dehydrated – 25 lb (11.3 kg)†

United States and Canada

Cat. No.	221173	Prepared Plates – Pkg. of 20*†
	221271	Prepared Plates – Ctn. of 100*†

Europe

Cat. No.	254027	Prepared Plates – Pkg. of 20*†
	254079	Prepared Plates – Ctn. of 120*†

Japan

Cat. No.	251173	Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*
†QC testing performed according to USP/EP/JP performance specifications.

Marine Agar 2216 • Marine Broth 2216

Intended Use

Marine Agar 2216 and Marine Broth 2216 are used for cultivating heterotrophic marine bacteria.

Summary and Explanation

Marine bacteria are present in nutrient sea water by the millions per mL and are essential to the life cycle of all marine flora and fauna. The enumeration and activity of marine bacteria are important to the food industry for the conservation of marine life. Marine Agar 2216 and Marine Broth 2216 are prepared according to the formula of ZoBell[1] The media contain all of the nutrients necessary for the growth of marine bacteria.

User Quality Control

Identity Specifications

Difco™ Marine Agar 2216

Dehydrated Appearance:	Light beige with a few dark particles, free flowing, homogeneous.
Solution:	5.51% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent to opalescent with slight precipitate.
Prepared Appearance:	Light amber, slightly opalescent to opalescent, may have a slight precipitate, may contain dark particles.
Reaction of 5.51% Solution at 25°C:	pH 7.6 ± 0.2

Difco™ Marine Broth 2216

Dehydrated Appearance:	Light beige with a few dark particles, free flowing, homogeneous.
Solution:	3.74% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent with precipitate.
Prepared Appearance:	Light amber, slightly opalescent with a precipitate.
Reaction of 3.74% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Difco™ Marine Agar 2216

Prepare the medium per label directions. Inoculate and incubate at 20-25°C for 40-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Vibrio fischeri	7744	10^2-10^3	Good
Vibrio harveyi	14126	10^2-10^3	Good

Difco™ Marine Broth 2216

Prepare the medium per label directions. Dispense 50 mL amounts in 250 mL Erlenmeyer flasks. Inoculate and incubate at 20-25°C on a shaker for 40-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Vibrio fischeri	7744	10^2-10^3	Good
Vibrio harveyi	14126	10^2-10^3	Good

The media contain minerals that nearly duplicate the major mineral composition of sea water,[2] in addition to peptone and yeast extract that provide a good source of nutrients.

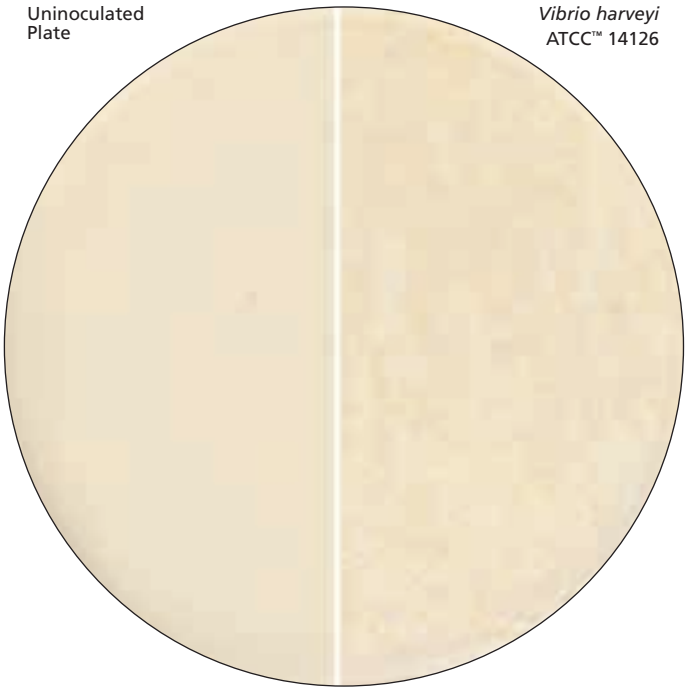
In the use of Marine Agar 2216, the conventional pour plate and spread plate techniques of enumeration are used. For the pour plate technique, the agar must be cooled to 42°C before inoculation because of the thermo-sensitive nature of most marine bacteria. In the spread plate technique, the agar is poured while hot and allowed to cool and solidify before inoculation. This latter method was reported by Buck and Cleverdon[3] to give higher counts than the pour plate method because of the increased growth of the thermo-sensitive bacteria.

Sizemore and Stevenson[4] used Marine Agar 2216 routinely as the upper nutrient layer of a marine agar-milk agar double-layer plate. This two layer plate was developed for isolating proteolytic marine bacteria. Marine Agar 2216 was also used in studies characterizing a marine bacterium associated with *Crassostrea virginica* (the Eastern Oyster).[5]

Principles of the Procedure

Peptone and yeast extract provide nitrogen, vitamins and minerals.

The high salt content helps to simulate sea water. Numerous minerals are also included to duplicate the major mineral composition of sea water. Agar is the solidifying agent.



Formulae

Difco™ Marine Agar 2216

Approximate Formula* Per Liter	
Peptone	5.0 g
Yeast Extract	1.0 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride	8.8 g
Sodium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide	0.08 g
Strontium Chloride	34.0 mg
Boric Acid	22.0 mg
Sodium Silicate	4.0 mg
Sodium Fluoride	2.4 mg
Ammonium Nitrate	1.6 mg
Disodium Phosphate	8.0 mg
Agar	15.0 g

Difco™ Marine Broth 2216

Approximate Formula* Per Liter	
Peptone	5.0 g
Yeast Extract	1.0 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride	5.9 g
Magnesium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide	0.08 g
Strontium Chloride	34.0 mg
Boric Acid	22.0 mg
Sodium Silicate	4.0 mg
Sodium Fluoride	2.4 mg
Ammonium Nitrate	1.6 mg
Disodium Phosphate	8.0 mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Marine Agar 2216 - 55.1 g;
   Difco™ Marine Broth 2216 - 37.4 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[3,4]

Expected Results

Refer to appropriate references and procedures for results.

References

1. ZoBell. 1941. J. Mar. Res. *4*:42.
2. Lyman and Fleming. 1940. J. Mar. Res. *3*:134.
3. Buck and Cleverdon. 1960. Limnol. Oceanogr. *5*:78.
4. Sizemore and Stevenson. 1970. Appl. Microbiol. *20*:991.
5. Weiner, Segall and Colwell. 1985. Appl. Environ. Microbiol. *49*:83.

Availability

Difco™ Marine Agar 2216

Cat. No. 212185 Dehydrated – 500 g

Difco™ Marine Broth 2216

Cat. No. 279110 Dehydrated – 500 g
214907 Dehydrated – 10 kg

Martin-Lewis Agar • Martin-Lewis Agar, Modified Martin-Lewis Agar (Gono-Pak) • Martin-Lewis Agar (JEMBEC™)

Intended Use

Martin-Lewis Agar and Martin-Lewis Agar, Modified are used for the isolation of pathogenic *Neisseria* from specimens containing mixed flora of bacteria and fungi.

Summary and Explanation

Thayer-Martin Selective Agar was developed for the primary isolation of *N. gonorrhoeae* and *N. meningitidis* from specimens containing mixed flora taken from the throat, vagina, rectum, and urethra.[1-3] Consisting of **BBL**™ Chocolate II Agar with vancomycin, colistin and nystatin, it is formulated to minimize the overgrowth of gonococci and meningococci by contaminants, to suppress the growth of saprophytic *Neisseria* species and to enhance the growth of pathogenic *Neisseria*.

Martin et al. modified Thayer-Martin Selective Agar by adding trimethoprim to produce Modified Thayer-Martin (MTM) Selective Agar.[4] A significantly greater number of positive gonococcal isolates from clinical specimens was reported as compared with Thayer-Martin Selective Agar due to the inhibition of swarming *Proteus* species.[4-6] Because of its improved performance, it is recommended over earlier formulations for the isolation of *N. gonorrhoeae*.[7] The original formula contained 20 g/L of agar and 1.5 g/L dextrose (in addition to the dextrose in the **IsoVitaleX**™ Enrichment). The agar concentration has been changed to approximately 12 g/L; the extra 1.5 g/L of dextrose has been eliminated since the lower dextrose content was found to improve the growth of *N. gonorrhoeae*. **BBL** MTM II was developed by careful selection and pretesting of raw materials to provide enhanced growth of gonococci as well as improved inhibition of *Candida* species.

Also recommended over earlier formulations is Martin-Lewis Agar, a further modification of the earlier formulations developed for the selective isolation of pathogenic *Neisseria*,

which is more inhibitory to gram-positive bacteria and yeasts than Thayer-Martin agars.[8,9] The concentration of vancomycin is increased from 3.0 mg/mL to 4.0 mg/mL for greater inhibition of gram-positive bacteria, and anisomycin is substituted for nystatin for improved inhibition of *Candida albicans*. This organism has been shown to inhibit *N. gonorrhoeae*.[10,11]

Martin-Lewis Agar, Modified has the same formulation as Martin-Lewis Agar except that the concentration of vancomycin has been reduced to 3.0 mg/mL.

Gono-Pak is the name given to a selective medium – resealable polyethylene bag – CO_2 generating tablet system described by Holston et al. for the isolation of *N. gonorrhoeae*. It was found to be comparable to the candle jar method for the isolation of *N. gonorrhoeae* from clinical specimens.[12,13] The Gono-Pak system obviates the need both for a separate carbon dioxide system and for transferring the specimen from the transport system to a culture plate. It has been reported to be superior to Transgrow (Modified Thayer-Martin Agar with a CO_2-enriched atmosphere in a bottle) as a transport system.[14]

The JEMBEC™* style plate was developed by John E. Martin, Jr., of the Centers for Disease Control in association with Ames Laboratories and was designed to provide a self-contained CO_2 environment through the use of a CO_2-generating tablet placed in a specially designed well provided in the plate.[15] The JEMBEC system is recommended for the growth and transportation of *N. gonorrhoeae* and has the advantage over other transport systems of obviating the necessity of transferring the specimen from the transport system to a culture plate.

**JEMBEC is a trademark of Miles Scientific.*

Principles of the Procedure

Martin-Lewis Agar is based on Chocolate II Agar which contains an improved GC Agar base, bovine hemoglobin and **BBL™IsoVitaleX™** Enrichment. The GC base contains nitrogenous nutrients in the form of casein and meat peptones, phosphate buffer to maintain pH and corn starch, which neutralizes toxic fatty acids that may be present in the agar. Hemoglobin provides X factor (hemin) for *Haemophilus* species. **IsoVitaleX** Enrichment is a defined supplement which provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

This selective medium contains the antimicrobial agents vancomycin, colistin, anisomycin (V-C-A Inhibitor) and trimethoprim, to suppress the normal flora. Vancomycin is active primarily against gram-positive bacteria. Colistin inhibits gram-negative bacteria, including *Pseudomonas* species, but is not active against *Proteus* species. Anisomycin inhibits yeasts. Trimethoprim inhibits *Proteus*.

In the Gono-Pak system, a tablet consisting of a mixture of citric acid and sodium bicarbonate is activated by the moisture (humidity) produced by the culture medium within the sealed plastic bag and generates CO_2 levels sufficient for the growth of *Neisseria gonorrhoeae* on the selective media provided with the system.[15]

In the JEMBEC system, a tablet consisting of a mixture of citric acid and sodium bicarbonate is placed in a well within the plate and is activated by the moisture (humidity) produced by the culture medium within the sealed plastic bag. The CO_2 levels generated are sufficient for the growth of *N. gonorrhoeae* on the selective media provided with the system.[15]

Procedure

Streak the specimen as soon as possible after it is received in the laboratory. If material is cultured directly from a swab, proceed as follows:[16]

1. Roll swab directly on the medium in a large "Z" to provide adequate exposure of swab to the medium for transfer of organisms.
2. Cross-streak the "Z" pattern with a sterile wire loop, preferably in the clinic. If not done previously, cross-streaking should be done in the laboratory.
3. Place the culture as soon as possible in an aerobic environment enriched with carbon dioxide.

   a. With the Gono-Pak System:

   Place inoculated plates in the polyethylene bag provided (one or two plates per bag). Cut off the corner of one foil-wrapped CO_2 tablet to expose the tablet and place in the bag. DO NOT ADD WATER TO THE TABLET.

   To seal the bag, simply press down on the "zipper" at the end of the bag with fingers and slide along to the opposite end. Be sure that the bag is sealed completely. After the bag is sealed, incubate in an inverted position (agar bed up) at 35°C for 18 to 48 hours.[7,17]

   To transport the culture after incubation, place the sealed Gono-Pak system in a suitable mailing or shipping container. Care should be taken to protect the culture from extreme heat or cold and to ensure delivery to the testing laboratory as rapidly as possible.

   b. With the JEMBEC System:

   With sterile forceps, remove a CO_2-generating tablet from its foil wrapper and place it in the specially designed well in the plate. Place inoculated plates in the polyethylene bag provided (one plate per bag). DO NOT ADD WATER TO THE TABLET. Seal the bag by pressing down on the "zipper" at the end of the bag with fingers and slide along to the opposite end. Be sure that the bag is sealed completely. After the bag is sealed, incubate in an inverted position (agar bed up) at 35°C for 18-48 hours.[7,17]

   To transport the culture after incubation, place the sealed JEMBEC system in a suitable mailing or shipping container. Care should be taken to protect the culture from extreme heat or cold and to ensure delivery to the testing laboratory as soon as possible.

   Transit time may be extended and viability and recovery enhanced by incubating before mailing. An incubation

time of 18-24 hours at 35 ± 2°C will enhance transport and recovery of organisms for definitive examinations immediately upon arrival in the laboratory.[9] Specimens should arrive at the laboratory within 48 hours.[9]

If confluent growth is obtained on the surface of the medium, subculture growth to Martin-Lewis Agar and nonselective Chocolate II Agar and pick isolated colonies from the subculture plates for further investigation.

If isolated colonies are present on the agar surface of the medium, pick colonies for Gram staining, oxidase testing and for carbohydrate fermentation studies.

4. Incubate at 35 ± 2°C and examine after overnight incubation and again after approximately 48 hours.
5. Subculture for identification of *N. gonorrhoeae* should be made within 18-24 hours. If shipped after incubation, colonies should be subcultured before performing biochemical identification tests in order to ensure that adequate viability is achieved.

Expected Results

Typical colonial morphology on Martin-Lewis Agar is as follows:

Neisseria gonorrhoeae ..... Small grayish-white to colorless, mucoid

Neisseria meningitidis ..... Medium to large, blue-gray, mucoid

Colonies may be selected for Gram staining, subculturing or other diagnostic procedures.

References

1. Martin, Billings, Hackney and Thayer. 1967. Public Health Rep. *82*:361.
2. Thayer and Martin. 1966. Pub. Health Rep. *81*:559.
3. Mitchell, Rhoden and Marcus. 1966. Am. J. Epidem. *83*:74.
4. Martin, Armstrong and Smith. 1974. Appl. Microbiol. *27*:802.
5. Center for Disease Control, January 2, 1975. Memorandum: recommendation to use the same medium, Modified Thayer-Martin (MTM), in both plates and bottles for the GC culture screening program. U.S. Public Health Service, Atlanta, Ga.
6. Seth. 1970. Br. J. Vener. Dis. *46*:201.
7. Evangelista and Beilstein. 1993. Cumitech 4A, Laboratory diagnosis of gonorrhea. Coord. ed., Abramson. American Society for Microbiology, Washington, D.C.
8. Martin and Lewis. 1977. Public Health Lab. *35*:53.
9. Knapp and Koumans. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
10. Hipp, Lawton, Chen and Gaafar. 1974. Appl. Microbiol. *27*:192.
11. Hipp, Lawton, Savage and Gaafar. 1975. J. Clin. Microbiol. *1*:476.
12. Holston, Hosty and Martin. 1974. Am. J. Clin. Pathol. *62*:558.
13. DeVaux, Evans, Arndt and Janda. 1987. J. Clin. Microbiol. *25*:571.
14. Lewis and Weisner. 1980. Lab Management. *18*:33.
15. Martin and Jackson. 1975. J. Am. Ven. Dis. Assoc. *2*:28.
16. Center for Disease Control. 1975. Criteria and techniques for the diagnosis of gonorrhea. U.S. Public Health Service, Atlanta, Ga.
17. Lewis. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Martin-Lewis Agar

Cat. No. 221557 Prepared Plates – Pkg. of 20*
221558 Prepared Plates – Ctn. of 100*

BBL™ Martin-Lewis Agar//Chocolate II Agar

Cat. No. 297060 Prepared Bi-Plate Dishes – Pkg. of 20*
297245 Prepared Bi-Plate Dishes – Ctn. of 100*

BBL™ Martin-Lewis, Modified//Chocolate II Agar

Cat. No. 298513 Prepared Bi-Plate Dishes – Pkg. of 20*
298206 Prepared Bi-Plate Dishes – Ctn. of 100*

BBL™ Martin-Lewis Agar (Gono-Pak)

Cat. No. 221793 Prepared Plates – Pkg. of 20*

BBL™ Martin-Lewis Agar (JEMBEC™)

Cat. No. 221804 Prepared Plates – Pkg. of 10*
299602 Prepared Plates (with white patient label on bottom) – Pkg. of 10*

**Store at 2-8°C.*

Maximum Recovery Diluent

Intended Use

Maximum Recovery Diluent is an isotonic diluent containing a low level of peptone used for maintaining the viability of organisms during dilution procedures.

Summary and Explanation

Standard methods for the microbiological examination of foodstuffs require sample dilution to be carried out accurately to estimate the number of microorganisms. Diluents consisting of sterile saline, phosphate buffer solutions and distilled water have all been shown to have a lethal action on a wide range of organisms.[1,2]

The presence of low levels of peptone in the diluent at a pH of 7.0 ± 0.2 affords protection for bacteria for at least one hour during the dilution stage.[3,4] The presence of peptone also allows accurate quantitative procedures to be performed with minimal reductions in viable count in the diluent.

Principles of Procedure

Low levels of peptone help protect organisms in the diluent. Sodium chloride maintains proper osmotic pressure.

User Quality Control

Identity Specifications

Difco™ Maximum Recovery Diluent

Dehydrated Appearance:	Cream to beige, free-flowing, homogeneous.
Solution:	0.95% solution, soluble in purified water. Solution is colorless, clear.
Prepared Appearance:	Colorless, clear.
Reaction of 0.95% Solution at 25°C:	pH 7.0 ± 0.2

Survival Test

Difco™ Maximum Recovery Diluent

Prepare the medium per label directions. Inoculate tubes with the test organism. At time zero and after 30 minutes at room temperature, subculture a loopful (0.01 mL) onto **Trypticase**™ Soy Agar with 5% Sheep Blood (TSA II) plates using the streak technique. Incubate plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AFTER 30 MINUTES
Escherichia coli	25922	10^3-10^4	No significant reduction
Staphylococcus aureus	25923	10^3-10^4	No significant reduction

Formula

Difco™ Maximum Recovery Diluent

Approximate Formula* Per Liter		
Peptone	1.0	g
Sodium Chloride	8.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 9.5 g of the powder in 1 L of purified water.
2. Dispense into final containers and cap loosely.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for dilution procedures when testing foods.[1-4]

Expected Results

Refer to appropriate references and procedures for results.

References

1. DeMello, Danielson and Kiser. 1951. J. Lab. Clin. Med. 37:579.
2. Gunter. 1954. J. Bacteriol. 67:628.
3. Straka and Stokes. 1957. Appl. Microbiol. 5:21.
4. Patterson and Cassells. 1963. J. Appl. Bacteriol. 26:493.

Availability

Difco™ Maximum Recovery Diluent

Cat. No. 218971 Dehydrated – 500 g

McClung Toabe Agar Base Egg Yolk Enrichment 50%

Intended Use

McClung Toabe Agar Base is used with Egg Yolk Enrichment 50% for isolating and detecting *Clostridium perfringens* in foods based on the lecithinase reaction.

Summary and Explanation

McClung and Toabe[1] formulated a medium for isolating *C. perfringens* from foods. With the addition of 50% egg yolk emulsion, *C. perfringens* and a few other *Clostridium* species show the lecithinase reaction.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients, but occurrences of food borne illness are usually associated with cooked meat or poultry products.[2] Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.[3] Enumerating the microorganism in food samples plays a role in epidemiological investigation of outbreaks of food borne illness.[2]

User Quality Control

Identity Specifications

Difco™ McClung Toabe Agar Base

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	7.5% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent, with a precipitate.
Prepared Appearance:	Plain – Light amber, opalescent with precipitate. With Egg Yolk Enrichment – Light yellow, opaque.
Reaction of 7.5% Solution at 25°C:	pH 7.6 ± 0.2

Difco™ Egg Yolk Enrichment 50%

Appearance:	Canary yellow, opaque liquid with a resuspendable precipitate.

Cultural Response

Difco™ McClung Toabe Agar Base with Egg Yolk Enrichment 50%

Prepare the medium with Egg Yolk Enrichment 50% per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. Incubate the clostridia anaerobically.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	LECITHINASE REACTION
Clostridium perfringens	12919	10^2-10^3	Good	Opaque halo
Clostridium perfringens	12924	10^2-10^3	Good	Opaque halo
Staphylococcus aureus	25923	10^2-10^3	Good	None
Staphylococcus epidermidis	14990	10^2-10^3	Good	None

Clostridium perfringens
ATCC™ 12919

Principles of the Procedure

McClung Toabe Agar Base contains peptone as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate source. Sodium chloride provides essential ions. Magnesium sulfate provides divalent cations and sulfate. Disodium phosphate and monopotassium phosphate maintain pH balance and provide a source of phosphates. Agar is the solidifying agent. Egg Yolk Enrichment 50% provides egg yolk lecithin. Lecithinase-producing clostridia, such as *C. perfringens*, hydrolyze the lecithin and produce opaque halos.

Formulae

Difco™ McClung Toabe Agar Base

Approximate Formula* Per Liter

Proteose Peptone	40.0	g
Dextrose	2.0	g
Disodium Phosphate	5.0	g
Monopotassium Phosphate	1.0	g
Sodium Chloride	2.0	g
Magnesium Sulfate	0.1	g
Agar	25.0	g

Difco™ Egg Yolk Enrichment 50%

Concentrated egg yolk emulsion.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ McClung Toabe Agar Base

1. Suspend 75 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense 90 mL amounts into flasks and autoclave at 121°C for 15 minutes.
4. Cool to 50°C and aseptically add 10 mL of Egg Yolk Enrichment 50% to each 90 mL of base. Mix thoroughly.
5. Dispense into sterile Petri dishes in approximately 15 mL amounts.
6. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Egg Yolk Enrichment 50%

Shake gently to resuspend the precipitate.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. McClung and Toabe. 1947. J. Bacteriol. *53*:139.
2. Labbe. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ McClung Toabe Agar Base

Cat. No.	294110	Dehydrated – 500 g

Difco™ Egg Yolk Enrichment 50%

Cat. No.	233471	Tube – 12 × 10 mL*
	233472	Bottle – 6 × 100 mL*

**Store at 2-8°C.*

McFarland Turbidity Standard No. 0.5

Intended Use

McFarland standards are used as turbidity standards in the preparation of suspensions of microorganisms. The McFarland 0.5 standard has particular application in the preparation of bacterial inocula for performing antimicrobial susceptibility testing.

Summary and Explanation

One of the earliest uses of turbidity for the estimation of bacterial populations was in the preparation of vaccines.[1] In 1907, McFarland developed a series of barium sulfate solutions to approximate the numbers of bacteria in solutions of equal turbidity, as determined by plate counts.[2,3]

The performance of susceptibility testing requires the use of standard inocula. The McFarland 0.5 standard is used for the preparation of inocula in standardized agar dilution, broth macro- and microdilution, disc diffusion and anaerobic organism susceptibility test procedures.[4-6]

Principles of the Procedure

Turbidity standards are prepared by mixing chemicals that precipitate to form a solution of reproducible turbidity.[3] McFarland standards are prepared by adding sulfuric acid to an aqueous solution of barium chloride, which results in the formation of a suspended barium sulfate precipitate.

The McFarland 0.5 standard corresponds approximately to a homogeneous *Escherichia coli* suspension of 1.5×10^8 cells per mL.[3]

Procedure

Vigorously agitate the turbidity standard on a mechanical vortex mixer just before use.

Using adequate light, compare the turbidity of a bacterial suspension to the turbidity standard by holding the tubes against a white background with contrasting horizontal black lines.

Alternatively, use the turbidity standard to standardize electrometric turbidimeters.

Expected Results

Use of the McFarland 0.5 standard will enable the preparation of standardized inocula for use in the performance of standardized antimicrobial susceptibility testing procedures.[4-6]

Availability

BBL™ McFarland Turbidity Standard No. 0.5

BAM CLSI COMPF

Cat. No. 297298 Prepared Tubes (K Tubes) – Pkg. of 10

References

1. Lorian (ed.). 1986. Antibiotics in laboratory medicine, 2nd ed. Williams & Wilkins, Baltimore, Md.
2. McFarland. 1907. J. Am. Med. Assoc. *49*:1176.
3. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
4. Clinical and Laboratory Standards Institute. 2006. Approved Standard: M7-A7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. CLSI, Wayne, Pa.
5. Clinical and Laboratory Standards Institute. 2006. Approved Standard: M2-A9. Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.
6. Clinical and Laboratory Standards Institute. 2007. Approved Standard: M11-A7. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 7th ed. CLSI, Wayne, Pa.

Micro Assay Culture Agar • Micro Inoculum Broth

Intended Use

Micro Assay Culture Agar is used for cultivating lactobacilli and other organisms used in microbiological assays.

Micro Inoculum Broth is used for preparing the inoculum of lactobacilli and other microorganisms used in microbiological assays of vitamins and amino acids.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins.

Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Micro Assay Culture Agar is used for maintaining stock cultures of lactobacilli and other test microorganisms. This medium is also used for general cultivation of lactobacilli.

Micro Inoculum Broth is used for cultivating lactobacilli and preparing the inoculum for microbiological assays.

Principles of the Procedure

Peptone provides nitrogen and amino acids in both Micro Assay Culture Agar and Micro Inoculum Broth. Yeast extract is a vitamin source. Dextrose is a carbon source. Monopotassium phosphate is a buffering agent. Polysorbate 80 acts as an emulsifier. Agar is the solidifying agent (Micro Assay Culture Agar).

User Quality Control

Identity Specifications

Difco™ Micro Assay Culture Agar

Dehydrated Appearance:	Light tan to tan, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 6.7 ± 0.2

Difco™ Micro Inoculum Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent, without precipitate.
Reaction of 3.7% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Micro Assay Culture Agar or Micro Inoculum Broth

Prepare the medium per label directions. Inoculate with test organisms. Incubate Micro Assay Culture Agar at 35 ± 2°C for 18-48 hours; incubate Micro Inoculum Broth at 35-37°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus hirae	8043	10^2-10^3	Good
Lactobacillus rhamnosus	7469	10^2-10^3	Good
Lactobacillus delbrueckii subsp. *lactis*	7830	10^2-10^3	Good
Lactobacillus plantarum	8014	10^2-10^3	Good

Formulae

Difco™ Micro Assay Culture Agar

Approximate Formula* Per Liter

Proteose Peptone No. 3	5.0	g
Yeast Extract	20.0	g
Dextrose	10.0	g
Monopotassium Phosphate	2.0	g
Polysorbate 80	0.1	g
Agar	10.0	g

Difco™ Micro Inoculum Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware used in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

Difco™ Micro Assay Culture Agar

1. Suspend 47 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense 10 mL amounts into 16-20 mm diameter tubes.
4. Autoclave at 121°C for 15 minutes.
5. Agitate tubes prior to solidification to disperse the flocculent precipitate.
6. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Micro Inoculum Broth

1. Dissolve 37 g of the powder in 1 L of purified water.
2. Dispense 10 mL quantities into tubes of 16-20 mm diameter.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Stock Cultures

1. Prepare stock cultures in triplicate on Micro Assay Culture Agar, inoculating tubes using a straight-wire inoculating needle.
2. Incubate tubes at 30-37°C for 18-24 hours.
3. Store at 2-8°C.
4. Transfer cultures at weekly or twice-monthly intervals.

Assay Inoculum

1. Subculture from a 16-24 hour stock culture of lactobacilli in Micro Assay Culture Agar into a 10 mL tube of Micro Inoculum Broth.
2. Incubate at 35-37°C for 16-24 hours or as specified in the assay procedure.
3. Centrifuge the culture and decant the supernatant.
4. Resuspend cells in 10 mL of sterile 0.9% NaCl solution or sterile single strength basal assay medium.
5. Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise indicated.
6. Dilute the washed suspension 1:100 with sterile 0.9% single strength basal assay medium or as indicated. Where applicable, adjust inoculum concentration according to limits specified in the references.[1,2]

For a complete discussion of vitamin assay methodology, refer to appropriate procedures.[1,2]

Expected Results

For test results on vitamin assay procedures, refer to appropriate procedures.[1,2]

Limitations of the Procedure

1. Test organisms used in assay procedures must be cultured and maintained on media recommended for this purpose.
2. Follow assay directions exactly. The age, preparation and size of inoculum are extremely important factors in obtaining a satisfactory assay result.
3. Although other media and methods may be used successfully for maintaining cultures and preparing inocula, uniformly good results will be obtained if the methods described are followed exactly.
4. Aseptic technique should be used throughout the microbiological assay procedure.
5. The use of altered or deficient media may create mutants having different nutritional requirements. Such organisms will not produce a satisfactory test response.

References

1. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed, online. AOAC International, Gaithersburg, Md.
2. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Micro Assay Culture Agar

Cat. No. 231920 Dehydrated – 500 g

Difco™ Micro Inoculum Broth

Cat. No. 211813 Dehydrated – 500 g

Microbial Content Test Agar

(See Tryptic Soy Agar with Lecithin and Polysorbate 80)

Middlebrook 7H9 Broth • Middlebrook 7H9 Broth with Glycerol • Middlebrook 7H9 Broth with Polysorbate 80 • Middlebrook ADC Enrichment

Intended Use

These media are used in qualitative procedures for the cultivation of mycobacteria.

Middlebrook 7H9 Broth dehydrated base (which may be supplemented with either glycerol or polysorbate 80) in combination with Middlebrook ADC Enrichment, and Middlebrook 7H9 Broth prepared tubes (containing ADC Enrichment) when supplemented with glycerol (2 mL/L) support the growth of mycobacteria, including *M. tuberculosis*. They are used primarily for growth of pure cultures of mycobacteria for use in laboratory studies.

These media and 7H9 Broth with Polysorbate 80 (0.5 mL/L) are used as subculture media for *Mycobacterium* species and in the preparation of inocula for drug susceptibility testing.

User Quality Control

Identity Specifications

Difco™ Middlebrook 7H9 Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	0.47% solution, soluble in purified water. Solution is very light amber, clear.
Prepared Appearance:	Colorless to very light amber, clear.
Reaction of 0.47% Solution at 25°C:	pH 6.6 ± 0.2

BBL™ Middlebrook ADC Enrichment

Appearance:	Very pale yellow, clear to trace hazy.
Reaction of Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Middlebrook 7H9 Broth with BBL™ Middlebrook ADC Enrichment

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for up to 21 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Mycobacterium tuberculosis H37Ra	25177	10^2-3×10^2	Good
Mycobacterium kansasii, Group I	12478	10^2-3×10^2	Good
Mycobacterium scrofulaceum, Group II	19981	10^2-3×10^2	Good
Mycobacterium intracellulare, Group III	13950	10^2-3×10^2	Good
Mycobacterium fortuitum, Group IV	6841	10^2-3×10^2	Good

Summary and Explanation

Middlebrook and co-workers developed the 7H9 broth base formulation during the same time period in which they devised the 7H10 agar base.[1-3] Both media types support the growth of mycobacterial species when supplemented with nutrients such as glycerol, oleic acid, albumin and dextrose, except for *M. bovis* which is inhibited by glycerol. The albumin and dextrose, along with sodium chloride and catalase are provided by the Middlebrook ADC Enrichment.

Middlebrook 7H9 Broth may be additionally supplemented with polysorbate 80 for improved growth.[4,5] Middlebrook broths are commonly used in the preparation of inocula for antimicrobial assays, biochemical tests (arylsufatase and tellurite reduction) and for maintenance of stock strains.[4-6]

Principles of the Procedure

The large number of inorganic salts in this medium provide substances essential for the growth of mycobacteria. Sodium citrate, when converted to citric acid, serves to hold certain inorganic cations in solution. The albumin acts as a protective agent by binding free fatty acids, which may be toxic to *Mycobacterium* species. In the enriched medium, the albumin is heat-treated to remove lipase, which may release fatty acids from polysorbate 80;[7] catalase destroys toxic peroxides that may be present in the medium; dextrose is an energy source; and sodium chloride provides essential electrolytes. Supplementation with glycerol or polysorbate 80 enhances the growth of mycobacteria.

Formulae

Difco™ Middlebrook 7H9 Broth

Approximate Formula* Per 900 mL	
Ammonium Sulfate	0.5 g
L-Glutamic Acid	0.5 g
Sodium Citrate	0.1 g
Pyridoxine	1.0 mg
Biotin	0.5 mg
Disodium Phosphate	2.5 g
Monopotassium Phosphate	1.0 g
Ferric Ammonium Citrate	0.04 g
Magnesium Sulfate	0.05 g
Calcium Chloride	0.5 mg
Zinc Sulfate	1.0 mg
Copper Sulfate	1.0 mg

BBL™ Middlebrook ADC Enrichment

Approximate Formula* Per Liter	
Sodium Chloride	8.5 g
Bovine Albumin (Fraction V)	50.0 g
Dextrose	20.0 g
Catalase	0.03 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precaution[8]

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Directions for Preparation from Dehydrated Product

1. Suspend 4.7 g of the powder in 900 mL of purified water (containing 2 mL glycerol or 0.5 g polysorbate 80, if desired).
2. Autoclave at 121°C for 10 minutes.
3. Aseptically add 100 mL of Middlebrook ADC Enrichment to the medium when cooled to 45°C.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Middlebrook 7H9 Broth with appropriate supplements is primarily used for growth of pure cultures of mycobacteria for use in laboratory studies. Place inoculated tubes in a **BD GasPak**™ EZ container operated with a **GasPak** EZ carbon dioxide generator sachet, or other suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C for up to 8 weeks. Keep the caps of the tubes loosened for at least 1 week to permit circulation of CO_2, but tighten the caps thereafter to prevent dehydration, loosening briefly once a week to replenish CO_2.

NOTE: Cultures from skin lesions suspected to be *M. marinum* or *M. ulcerans* should be incubated at 25-33°C for primary incubation. Cultures suspected to contain *M. avium* or *M. xenopi* exhbit optimum growth at 40-42°C.[9] Incubate a duplicate culture at 35-37°C.[9]

Expected Results

Cultures should be read within 5-7 days after inoculation and once a week thereafter for up to 8 weeks.

Mycobacterial growth from the broth tubes can be utilized for additional laboratory test procedures as required.

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

1. Middlebrook. 1955. Fitzsimmons Army Hospital Report No. 1, Denver, Colo.
2. Middlebrook and Cohn. 1958. Am. J. Public Health. *48*:844.
3. Middlebrook, Cohn and Schaefer. 1954. Am. Rev. Tuberc. *70*:852.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Washington (ed.). 1985. Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
6. Metchock, Nolte and Wallace. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Middlebrook, Cohn, Dye, Russel and Levy. 1960. Acta. Tuberc. Scand. *38*:66.
8. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
9. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta, Ga.

Availability

Difco™ Middlebrook 7H9 Broth

AOAC SMWW

Cat. No. 271310 Dehydrated – 500 g

BBL™ Middlebrook 7H9 Broth

BS12 CMPH2 MCM9

Cat. No. 295939 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*

BBL™ Middlebrook 7H9 Broth with Glycerol

AOAC SMWW

Cat. No. 221832 Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*

BBL™ Middlebrook 7H9 Broth with Polysorbate 80

Cat. No. 297151 Prepared Tubes, 5 mL (C Tubes) – Pkg. of 10*

BBL™ Middlebrook ADC Enrichment

AOAC SMWW

Cat. No. 212352 Bottle, 100 mL – Pkg. of 6*

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

**Store at 2-8°C.*

Middlebrook 7H10 Agar • Middlebrook and Cohn 7H10 Agar • Middlebrook OADC Enrichment

Intended Use

Middlebrook and Cohn 7H10 Agar, when supplemented with Middlebrook OADC Enrichment, is used in qualitative procedures for the isolation and cultivation of mycobacteria.

Summary and Explanation

Over the years, many culture media have been devised for the cultivation of mycobacteria. The early ones were egg-based formulations and included Lowenstein-Jensen Medium and

User Quality Control

Identity Specifications

Difco™ Middlebrook 7H10 Agar

Dehydrated Appearance:	Light beige to light beige with slight green tint, free-flowing, homogeneous.
Solution:	1.9% solution, soluble in purified water upon boiling. Solution is light to medium amber with slight green tint, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 1.9% Solution at 25°C:	pH 6.6 ± 0.2

BBL™ Middlebrook OADC Enrichment

Appearance:	Very pale yellow, clear to trace hazy.
Reaction of Solution at 25°C:	pH 6.9 ± 0.2

Uninoculated Tube

Mycobacterium fortuitum ATCC™ 6841

Cultural Response

Difco™ Middlebrook 7H10 Agar with BBL™ Middlebrook OADC Enrichment

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under approximately 3-5% CO_2 for up to 21 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-2×10^3	Partial inhibition
Mycobacterium tuberculosis H37Ra	25177	10^2-3×10^2	Good
Mycobacterium kansasii, Group I	12478	10^2-3×10^2	Good
Mycobacterium scrofulaceum, Group II	19981	10^2-3×10^2	Good
Mycobacterium intracellulare, Group III	13950	10^2-3×10^2	Good
Mycobacterium fortuitum, Group IV	6841	10^2-3×10^2	Good

Petragnani Medium. Dubos and Middlebrook were instrumental in the development of a number of formulations which contained oleic acid and albumin as key ingredients to aid in the growth of the tubercle bacilli and to protect the organisms against a variety of toxic agents.[1] Subsequently, Middlebrook and Cohn improved the formulation of oleic acid-albumin agar and obtained faster, more luxuriant growth of *Mycobacterium* species on their medium designated as 7H10.[2,3] The oleic acid and bovine albumin, along with sodium chloride, dextrose and catalase, are provided by the Middlebrook OADC Enrichment.

It has been reported that the 7H10 medium tends to grow fewer contaminants than the egg-based media commonly used for the cultivation of mycobacteria.[4]

Prepared plates of the complete medium are deep-filled to reduce the effects of drying during prolonged incubation.

Principles of the Procedure

Middlebrook and Cohn 7H10 Agar Base contains a variety of inorganic salts that provide substances essential for the growth of mycobacteria. The sodium citrate, when converted to citric acid, serves to hold certain inorganic cations in solution. Glycerol is an abundant source of carbon and energy.

Supplementation of the agar base is required in order to obtain mycobacterial growth. In the enriched medium, sodium chloride maintains osmotic equilibrium; oleic acid, as well as other long chain fatty acids, can be utilized by tubercle bacilli and plays an important role in the metabolism of mycobacteria; the primary effect of albumin is that of protection of the tubercle bacilli against toxic agents and, therefore, it enhances their recovery on primary isolation; dextrose is an energy source; and catalase destroys toxic peroxides that may be present in the medium. Partial inhibition of bacteria is achieved by the presence of the malachite green dye.

Formulae

Difco™ Middlebrook 7H10 Agar

Approximate Formula* Per 900 mL		
Ammonium Sulfate	0.5	g
Monopotassium Phosphate	1.5	g
Disodium Phosphate	1.5	g
Sodium Citrate	0.4	g
Magnesium Sulfate	25.0	mg
Calcium Chloride	0.5	mg
Zinc Sulfate	1.0	mg
Copper Sulfate	1.0	mg
L-Glutamic Acid (sodium salt)	0.5	g
Ferric Ammonium Citrate	0.04	g
Pyridoxine Hydrochloride	1.0	mg
Biotin	0.5	mg
Malachite Green	250.0	µg
Agar	15.0	g

BBL™ Middlebrook OADC Enrichment

Approximate Formula* Per Liter		
Sodium Chloride	8.5	g
Dextrose	20.0	g
Bovine Albumin (Fraction V)	50.0	g
Catalase	0.03	g
Oleic Acid	0.6	mL

**Adjusted and/or supplemented as required to meet performance criteria.*

Precaution[9]

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Directions for Preparation from Dehydrated Product

1. Suspend 19 g of the powder in 900 mL of purified water containing 5 mL of glycerol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes.
4. Aseptically add 100 mL of Middlebrook OADC Enrichment to the medium when cooled to 50-55°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[5] N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. These reagents are provided in the **BD MycoPrep**™ Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[5-8]

Following inoculation, keep plates shielded from light and place plates, medium side down, in a **BD GasPak**™ EZ container operated with a **GasPak** EZ disposable carbon dioxide generator sachet, or other suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C.

Tubes should be incubated in a slanted position at a 5° angle, permitting incubation of slant surfaces in a horizontal plane. Tubes and bottles should have screw caps loose for at least 1 week to permit circulation of carbon dioxide for the initiation of growth. Thereafter, to prevent dehydration, tighten caps; loosen briefly once a week. Stand tubes upright if space is a problem.

NOTE: Cultures from skin lesions suspected to be *M. marinum* or *M. ulcerans* should be incubated at 25-33°C for primary incubation; cultures suspected to contain *M. avium* or *M. xenopi* exhibit optimum growth at 40-42°C.[5] Incubate a duplicate culture at 35-37°C.[5]

Expected Results

Cultures should be read within 5-7 days after inoculation and once a week thereafter for up to 8 weeks.

For reading plates or bottles, invert the containers on the stage of a dissecting microscope. Read at 10-60× with transmitted light. Scan rapidly at 10-20× for the presence of colonies. Higher magnification (30-60×) is helpful in observing colony morphology; i.e., serpentine cord-like colonies.

Record observations:[5]

1. Number of days required for colonies to become macroscopically visible.
2. Number of colonies (plates and bottles):
   No colonies = Negative
   Less than 50 colonies = Actual count
   50-100 colonies = 1+
   100-200 colonies = 2+
   Almost confluent (200-500) = 3+
   Confluent (more than 500) = 4+
3. Pigment production
   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Acid-fast-stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.[5]

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

1. Dubos and Middlebrook. 1947. Am. Rev. Tuberc. *56*:334.
2. Middlebrook and Cohn. 1958. Am. J. Pub. Health. *48*:844.
3. Middlebrook, Cohn, Dye, Russell and Levy. 1960. Acta Tuberc. Scand. *38*:66.
4. Kubica and Dye. 1967. Laboratory methods for clinical and public health mycobacteriology. PHS Publication No. 1547. U.S. Government Printing Office, Washington, D.C.
5. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta, Ga.
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7. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
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9. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication NO. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Difco™ Middlebrook 7H10 Agar

EP

Cat. No. 262710 Dehydrated – 500 g

BBL™ Middlebrook and Cohn 7H10 Agar

BS12 CMPH2 EP MCM9

United States and Canada

Cat. No. 221174 Prepared Plates (Deep Fill) – Pkg. of 20*
295964 Prepared **I Plate**™ Dishes (Middlebrook 7H10 Agar and Middlebrook 7H10 Agar) – Pkg. of 20*
220958 Prepared Slants, (A Tubes) – Pkg. of 10*
220959 Prepared Slants, (A Tubes) – Ctn. of 100*
297448 Prepared Slants, (C Tubes) – Pkg. of 10*
297396 Prepared Slants, (C Tubes) – Ctn. of 100*
297274 Prepared 1 oz. Transgrow-style Bottles – Ctn. of 100*

Europe

Cat. No. 254520 Prepared Plates – Pkg. of 20*

BBL™ Middlebrook OADC Enrichment

Cat. No. 212240 Bottle, 100 mL – Pkg. of 6*
212351 Bottle – 500 mL*

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

**Store at 2-8°C.*

Milk Agar

Intended Use

Milk Agar is recommended by the British Standards Institute[1] for the enumeration of microorganisms in liquid milk, ice cream, dried milk and whey.

Summary and Explanation

Liquid milk is a highly perishable foodstuff with a shelf life of only 5-10 days after pasteurization. Contamination of raw milk may arise from either the soiled or diseased udder or inadequately cleaned milking or storage equipment. Bovine mastitis or udder inflammation may cause contamination with *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* or, more rarely, *Yersinia enterocolitica* and *Leptospira* species. Excretion of these organisms can increase the bulk milk count by 10^5 organisms/mL.

User Quality Control

Identity Specifications

Difco™ Milk Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.2% solution, soluble in purified water upon boiling. Solution is light amber, clear to slightly opalescent, no significant precipitate.
Prepared Appearance:	Light amber, opalescent, no significant precipitate.
Reaction of 2.2% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

Difco™ Milk Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 30 ± 2°C for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus rhamnosus	9595	30-300	Good
Lactococcus lactis	19435	30-300	Good
Staphylococcus aureus	25923	30-300	Good
Streptococcus thermophilus	19258	30-300	Good

Poor cleaning of the milking equipment may cause contamination with micrococci, streptococci, coliforms or heat resistant *Bacillus* strains, giving an increase of the bulk milk count of >5 × 10^4 organisms/mL. Spoilage of pasteurized or raw milk by proteolytic psychrotrophic bacteria can occur on prolonged storage below 7°C.

Milk Agar conforms to the EEC Commission for the examination of ice cream.[2] Milk Agar is recommended for performing plate count tests on milks, rinse waters and dairy products.[3]

Principles of Procedure

Peptone and yeast extract provide essential nutrients while skim milk powder is a source of casein. Dextrose is the carbon energy source. Agar is the solidifying agent.

Proteolytic bacteria will be surrounded by a clear zone from the conversion of casein into soluble nitrogenous compounds.[1]

Formula

Difco™ Milk Agar

Approximate Formula* Per Liter

Tryptone	5.0	g
Yeast Extract	2.5	g
Dextrose	1.0	g
Skim Milk Powder (antibiotic free)	1.0	g
Agar	12.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 22 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Total counts may be carried out using either pour plates or surface counting techniques.

1. Prepare milk dilutions of 1/10, 1/100, 1/1,000 in 1/4-strength Ringer's solution. Use this inoculum within 15 minutes.
2. Pour Plates: Pipette 1 mL of each dilution into Petri dishes. Add 10-12 mL of molten Milk Agar, cooled to 45°C, and mix thoroughly.
   Spread Plates: Spread 1 mL of milk dilution over the surface of the solidified medium in a Petri dish.
3. Incubate at 30 ± 2°C for 72 hours.

Expected Results

Select plates containing 10-300 colonies. Results are expressed as colonies per mL of product tested.

Proteolytic psychrotrophic colonies may be enhanced by flooding the plates with a solution of 1% hydrochloric acid or 10% acetic acid. Pour off the excess acid solution and count the colonies surrounded by clear zones.

References

1. Methods of microbiological examination for dairy purposes. Diluents, media and apparatus and their preparation and sterilisation. BS4285, Sec. 1.2.
2. Klose. 1968. Susswaren. *14*:778.
3. Ministry of Health. 1937. Bacteriological tests for graded milk. Memo 139/Foods. H.M.S.O., London, England.

Availability

Difco™ Milk Agar

Cat. No. 218591 Dehydrated – 500 g

Minerals Modified Glutamate Broth

Intended Use

Minerals Modified Glutamate Broth is used for enumerating coliform organisms in water.

Summary and Explanation

Gray[1] described a simple formate-lactose-glutamate medium that could be used as an alternative to MacConkey Broth for the presumptive identification of coliform bacteria in water. Gray's original medium gave fewer false positive results than MacConkey Broth, was suitable for use at 44°C and gave low volumes of gas.

The medium was improved[2] by the addition of ammonium chloride that, by replacing ammonium lactate, resulted in a doubling of the gas volume. The addition of B-complex vitamins, certain amino acids and magnesium ions resulted in an increased rate of fermentation. Comparative trials of the modified glutamate medium and MacConkey Broth[3] with chlorinated and unchlorinated waters showed that Gray's Minerals Modified Glutamate Broth gave significantly higher numbers of positive results (acid and gas production) for coliform organisms and *Escherichia coli*. This was especially apparent after 48 hours of incubation but was also clearly seen with unchlorinated water samples after only 24 hours incubation. For chlorinated water samples, results with the two media were comparable. After 18-24 hours incubation, Minerals Modified Glutamate Medium gave significantly fewer false positive reactions. *Clostridium perfringens*, a common cause of false positive reactions in MacConkey media, is unable to grow in a minimal-glutamate based medium.

A major feature of Minerals Modified Glutamate Medium is its superiority in initiating growth of *Escherichia coli* after exposure to chlorine when incubated for 48 hours. In view of the known resistance to chlorination of some viruses, the ability to isolate coliform bacteria that survive marginal chlorination provides an additional safety factor in water treatment.

In a comparison to Lauryl Tryptose Lactose Broth[4], Minerals Modified Glutamate Medium gave superior isolation of *Escherichia coli* after 48 hours incubation by the multiple tube method, especially in waters containing small numbers of organisms. Minerals Modified Glutamate Medium is the medium of choice for the detection of fecal contamination in chlorinated drinking water supplies in Great Britain.[4]

Abbiss et al.[5] compared Minerals Modified Glutamate Medium and three other enrichment broths for the enumeration of coliform organisms present in soft cheese, cooked meat and patè. Minerals Modified Glutamate Medium was superior in sensitivity to Lauryl Sulfate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth.

Minerals Modified Glutamate Broth has been used in the modified direct plate method for enumeration of *Escherichia coli* biotype 1 in foods.[6] According to this method, 15 grams of agar are added per liter of single strength broth before autoclaving. The medium is poured in 12-15 mL amounts into sterile Petri dishes. This resuscitation agar is used for the recovery of damaged cells from frozen or dried foodstuffs.

Principles of the Procedure

Sodium glutamate and sodium formate are the basis of a defined minimal medium for the enumeration of coliform organisms in water. Lactose is the carbohydrate source in Minerals Modified Glutamate Broth. The addition of B-complex vitamins, certain amino acids and magnesium ions allows an increased rate of fermentation. Phosphate acts as a buffering agent. The addition of ammonium chloride allows increased gas production by the test organism. Bromocresol purple is present as a pH indicator.

User Quality Control

Identity Specifications

Difco™ Minerals Modified Glutamate Broth

Dehydrated Appearance:	Off-white to beige, free flowing, homogeneous.
Solution:	1.77% solution with 0.25% ammonium chloride, soluble in purified water on gentle warming. Solution is purple, clear.
Prepared Appearance:	Purple, clear.
Reaction of 1.77% Solution at 25°C:	pH 6.7 ± 0.1 (containing 0.25 g of ammonium chloride per 100 mL)

Cultural Response

Difco™ Minerals Modified Glutamate Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID	GAS
Enterobacter aerogenes	13048	30-300	Good	+ (yellow)	+
Enterococcus faecalis	19433	10^3	None	–	–
Escherichia coli	25922	30-300	Good	+ (yellow)	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	–	–

Formula

Difco™ Minerals Modified Glutamate Broth

Approximate Formula* Per Liter

Sodium Glutamate	6.4 g
Lactose	10.0 g
Sodium Formate	0.25 g
L-Cystine	0.02 g
L(-) Aspartic Acid	24.0 mg
L(+) Arginine	0.02 g
Thiamine	1.0 mg
Nicotinic Acid	1.0 mg
Pantothenic Acid	1.0 mg
Magnesium Sulfate Heptahydrate	0.1 g
Ferric Ammonium Citrate	0.01 g
Calcium Chloride Dihydrate	0.01 g
Dipotassium Phosphate	0.9 g
Bromocresol Purple	0.01 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 17.7 g of the powder in 1 L of purified water (or in 500 mL to prepare a double strength medium).
2. Add 2.5 g of ammonium chloride.
3. Mix well with gentle heating to dissolve.
4. Distribute into tubes and place an inverted fermentation tube in each tube.
5. Autoclave at 115-116°C for 10 minutes.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The multiple tube method is used for the enumeration of *Escherichia coli* and coliform organisms using Minerals Modified Glutamate Broth.

For good quality water, inoculate the water sample into the medium in the following volumes:

1. 50 mL of sample into 50 mL of double-strength medium;
2. 5 × 10 mL of sample into 5 × 10 mL of double-strength medium.

For more polluted waters, inoculate the water sample into the medium in the following volumes:

1. 5 × 1 mL of sample into 5 × 5 mL of single-strength medium;
2. 5 × 1 mL of a 1:10 dilution of the sample into 5 × 5 mL of single-strength medium.

Incubate the tubes at 35 ± 2°C. Examine after 18-24 hours incubation and again at 48 hours.

Expected Results

All tubes demonstrating acid production, indicated by the medium turning yellow, and gas, either in the inverted fermentation vial or by effervescence on shaking, may be regarded as presumptive positive reactions. Each presumptive positive tube should be confirmed in Brilliant Green Bile 2%, as well as with additional biochemical tests.

The most probable number of organisms in 100 mL of the original water sample can be calculated using the following table.[7]

QUANTITY OF WATER IN EACH TUBE	50 mL	10 mL	MOST PROBABLE NUMBER (MPN)
NUMBER OF TUBES USED	1	2	OF COLIFORMS IN 100 mL IN SAMPLE
	0	0	0
Number of Tubes	0	1	1
Giving Positive	0	2	2
Reaction	0	3	4
	0	4	5
	0	5	7
	1	0	2
	1	1	3
	1	2	6
	1	3	9
	1	4	16
	1	5	+18

Limitations of the Procedure

1. The performance of the medium is significantly affected by pH. Avoid overheating the broth. Check the pH of each lot before proceeding with testing.
2. Due to the nutritional requirements of the organisms, some organisms other than coliform bacteria may grow in the medium with production of acid and gas. Test all presumptive-positive tubes to confirm the presence of *Escherichia coli*.

References

1. Gray. 1959. J. Hyg., Camb. *57*:249.
2. Gray. 1964. J. Hyg., Camb. *62*:495.
3. P. H. L. S. Standing Committee on the Bacteriological Examination of Water Supplies. 1968. J. Hyg., Camb. *65*:67.
4. Joint Committee of the P. H. L. S. and the Standing Committee of Analysts. 1980. J. Hyg., Camb. *85*:35.
5. Abbiss, Wilson, Blood and Jarvis. 1981. J. Appl. Bact. *51*:121.
6. Holbrook, Anderson and Baird-Parker. 1980. Food Technol. Aust. *32*:78.
7. Departments of the Environment, Health & Social Security, and P.H.L.S. 1982. The bacteriological examination of drinking water supplies. Report on Public Health and Medical Subjects No. 71., H.M.S.O., London, England.

Availability

Difco™ Minerals Modified Glutamate Broth

Cat. No. 218501 Dehydrated – 500 g

Minimal Agar Davis
Minimal Broth Davis without Dextrose

Intended Use

Minimal Agar Davis is used for isolating and characterizing nutritional mutants of *Escherichia coli*.

Minimal Broth Davis without Dextrose is used with added dextrose in isolating and characterizing nutritional mutants of *Escherichia coli* and *Bacillus subtilis*.

Summary and Explanation

Lederberg[1] described the Davis formulation for Minimal Agar Davis. Minimal Broth Davis without Dextrose is the same formulation without dextrose and agar. Both media support the growth of nutritional mutants of *E. coli* while Minimal Broth Davis without Dextrose with added dextrose also supports the growth of nutritional mutants of *B. subtilis*.

Lederberg[1] described two techniques for isolating nutritional mutants of *E. coli*, one by random isolation and the other by delayed enrichment. Both Lederberg[1] and Davis[2] described a third technique using penicillin. Nutritional mutants of *B. subtilis* can be isolated by these three techniques and by a modification of the penicillin technique described by Nester, Schafer and Lederberg.[3]

After the mutants are isolated, they are characterized biochemically by growth in minimal broth supplemented with specific growth factors or groups of growth factors. It is generally best to classify mutants according to their requirements for amino acids, vitamins, nucleic acids or other substances. This is done by supplementing the minimal medium with Vitamin Assay Casamino Acids plus tryptophan, or a mixture of water soluble vitamins, alkaline-hydrolyzed yeast, nucleic acid or yeast extract, depending on the particular mutants desired. The supplemented minimal broth is inoculated with a slightly turbid suspension of the mutant colonies and incubated for 24 hours at 35°C. Growth with Vitamin Assay Casamino Acids indicates a vitamin requirement. When a major growth factor group response is obtained, the characterization is carried further by the same general procedure to subgroups and finally to individual growth substances.

Principles of the Procedure

Minimal Agar Davis and Minimal Broth Davis without Dextrose contain citrate and phosphates as buffers. Ammonium sulfate is the nitrogen source. Magnesium is a cofactor for many metabolic reactions. Minimal Agar Davis contains dextrose as the carbohydrate energy source. Agar is the solidifying agent.

Formulae

Difco™ Minimal Agar Davis

Approximate Formula* Per Liter

Dextrose	1.0	g
Dipotassium Phosphate	7.0	g
Monopotassium Phosphate	2.0	g
Sodium Citrate	0.5	g
Magnesium Sulfate	0.1	g
Ammonium Sulfate	1.0	g
Agar	15.0	g

Difco™ Minimal Broth Davis without Dextrose

Consists of the same ingredients without the dextrose and agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Minimal Agar Davis

1. Suspend 26.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Minimal Broth Davis without Dextrose

1. Dissolve 10.6 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. If desired, aseptically add 10 mL of 10% dextrose solution at room temperature. Mix thoroughly.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Random Technique

1. Irradiate a cell suspension of wild type *E. coli*.
2. Dilute the suspension 100-500×.
3. Culture on a complete agar medium containing all the necessary growth requirements.
4. Incubate the cultures at 35 ± 2°C for 24 hours.

User Quality Control

Identity Specifications

Difco™ Minimal Agar Davis

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.66% solution, soluble in purified water upon boiling. Solution is medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Medium amber, very slightly to slightly opalescent.
Reaction of 2.66% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ Minimal Broth Davis without Dextrose

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	1.06% solution, soluble in purified water. Solution is colorless, clear.
Prepared Appearance:	Colorless, clear.
Reaction of 1.06% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Minimal Agar Davis

Prepare the medium per label directions. Inoculate by the pour plate method and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	6883	10^2-10^3	Good
Escherichia coli	9637	10^2-10^3	Good

Difco™ Minimal Broth Davis without Dextrose

Prepare the medium per label directions with the addition of 1% dextrose. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10^2-10^3	Good
Escherichia coli	6883	10^2-10^3	Good
Escherichia coli	9637	10^2-10^3	Good

5. Select isolated colonies and inoculate into Minimal Broth Davis and a nutritionally complete broth.
6. Incubate at 35 ± 2°C for 24 hours.
7. Observe growth in both media.

Delayed Enrichment Method

1. Prepare plates of Minimal Agar Davis by pouring a 15-20 mL base layer in a 95 mm sterile Petri dish followed by a 5 mL seed layer.
2. Inoculate with a diluted irradiated *E. coli* suspension.
3. Pour a 5-10 mL layer of uninoculated Minimal Agar Davis over the seed layer.
4. Incubate for 24 hours or longer to allow for the growth of prototroph cells (wild type cells).
5. Pour a layer of a complete agar medium over the minimal agar medium to develop the mutant cells.
6. Incubate at 35 ± 2°C for 6-12 hours.

Penicillin Method

1. Wash an irradiated *E. coli* suspension with sterile saline and dilute to 20 × the original volume in sterile minimal broth.
2. Dispense into tubes in desired amounts.
3. Add freshly prepared penicillin to each tube to give a final concentration of 200 units per mL.
4. Incubate at 35 ± 2°C for 4-24 hours on a shaker.
5. Spread 0.1 mL, 0.01 mL and 0.001 mL samples onto complete agar plates.
6. Incubate at 35 ± 2°C for 24 hours.
7. Select isolated colonies and test for growth in minimal broth.

Bacillus subtilis Method

1. Grow cultures of *Bacillus subtilis* in Antibiotic Medium 3 at 35 ± 2°C for 18 hours.
2. Centrifuge to sediment the cells.
3. Aseptically decant the supernatant fluid.
4. Resuspend the cells in minimal medium and centrifuge.
5. Decant the supernatant and resuspend the pellet in minimal medium to give a cell concentration of about 2×10^8 cells per mL.
6. Irradiate the suspension with a low pressure mercury ultraviolet lamp for a sufficient time to give a cell survival of 1×10^4 cells per mL.
7. Incubate the suspension at room temperature for 4-18 hours in the minimal medium with appropriate substances added to allow for the growth of desired mutants.
8. Wash the culture in sterile minimal medium.
9. Centrifuge and resuspend in the same medium.
10. Dilute 1 to 10 with sterile minimal medium.
11. Let stand for 60 minutes to starve the mutants.
12. Add penicillin to give a concentration of 2,000 units per mL.
13. Incubate 15 minutes.
14. Plate the culture on nutrient agar for colony isolation.
15. Identify the nutrition mutants by transferring colonies by replicate plating onto plates of minimal agar which has been supplemented with the appropriate nutritional substances.

Expected Results

Random Technique

Growth in the nutritionally complete medium and no growth in the Minimal Broth indicates a mutant.

Delayed Enrichment Method

Mutant colonies will grow as small colonies after the addition of the complete medium which diffuses through the Minimal Agar.

Penicillin Method

Mutant colonies grow after the addition of penicillin.

Bacillus subtilis Method

Mutant colonies grow on Nutrient Agar after the addition of penicillin.

Limitation of the Procedure

Strains vary in their sensitivity to penicillin. Adjustments to the time of treatment and concentration of penicillin may be necessary.[1]

References

1. Lederberg. 1950. Methods in Med. Res. *3*:5.
2. Davis. 1949. Proc. Natl. Acad. Sci. *35*:1.
3. Nester, Schafer and Lederberg. 1963. Genetics *48*:529.

Availability

Difco™ Minimal Agar Davis

Cat. No. 254410 Dehydrated – 500 g

Difco™ Minimal Broth Davis without Dextrose

Cat. No. 275610 Dehydrated – 500 g

Mitis Salivarius Agar Tellurite Solution 1%

Intended Use

Mitis Salivarius Agar is used with Tellurite Solution 1% in isolating *Streptococcus mitis*, *S. salivarius* and enterococci, particularly from grossly contaminated specimens.

Summary and Explanation

S. mitis, *S. salivarius* and *Enterococcus* species are part of the normal human flora. *S. mitis* and *S. salivarius* are known as viridans streptococci. These organisms play a role in cariogenesis and infective endocarditis and cause an increasing number of bacteremias.[1] Enterococci cause urinary tract infections, wound infections and bacteremia.[2] These organisms can colonize the skin and mucous membranes.

Chapman[3-5] investigated methods for isolating streptococci and formulated Mitis Salivarius Agar. The medium facilitates isolation of *S. mitis* (*Streptococcus viridans*), *S. salivarius* (nonhemolytic streptococci) and enterococci from mixed cultures.[6]

Principles of the Procedure

Mitis Salivarius Agar contains peptones as sources of carbon, nitrogen, vitamins and minerals. Dextrose and saccharose are carbohydrate sources. Crystal violet and potassium tellurite (from Tellurite Solution 1%) inhibit most gram-negative bacilli and most gram-positive bacteria except streptococci. Trypan blue gives the colonies a blue color. Agar is the solidifying agent.

Formulae

Difco™ Mitis Salivarius Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	6.0	g
Proteose Peptone No. 3	9.0	g
Proteose Peptone	5.0	g
Dextrose	1.0	g
Saccharose	50.0	g
Dipotassium Phosphate	4.0	g
Trypan Blue	75.0	mg
Crystal Violet	0.8	mg
Agar	15.0	g

BBL™ Tellurite Solution 1%

Sterile 1% solution of Potassium Tellurite.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 90 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Add 1 mL of Tellurite Solution 1%. DO NOT HEAT THE COMPLETE MEDIUM.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

S. mitis produces small or minute blue colonies. These colonies may become easier to distinguish with longer incubation. *S. salivarius* produces blue, smooth or rough "gum drop" colonies, 1-5 mm in diameter depending on the number of colonies on the plate. *Enterococcus* species form dark blue or black, shiny, slightly raised, 1-2 mm colonies.

Limitations of the Procedure

1. If coliforms grow on the medium, they produce brown colonies.
2. Molds will grow on the medium after two days incubation.
3. *Erysipelothrix rhusiopathiae* produces colorless, circular, convex colonies.
4. Beta-hemolytic streptococci produce colonies that resemble *S. mitis*.

References

1. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology. Washington, D.C.
2. Facklam, Sahm and Teixeira. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Chapman. 1944. J. Bacteriol. *48*:113.
4. Chapman. 1946. Am. J. Dig. Dis. *13*:105.
5. Chapman. 1947. Trans. N.Y. Acad. Sci. (Series 2) *10*:45.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Mitis Salivarius Agar

Cat. No. 229810 Dehydrated – 500 g

BBL™ Tellurite Solution 1%

Cat. No. 211917 Tube – 20 mL

User Quality Control

Identity Specifications

Difco™ Mitis Salivarius Agar

Dehydrated Appearance:	Bluish-beige, free-flowing, homogeneous.
Solution:	9.0% solution, soluble in purified water upon boiling. Solution is deep royal blue, very slightly opalescent.
Prepared Appearance:	Deep royal blue, slightly opalescent.
Reaction of 9.0% Solution at 25°C:	pH 7.0 ± 0.2

BBL™ Tellurite Solution 1%

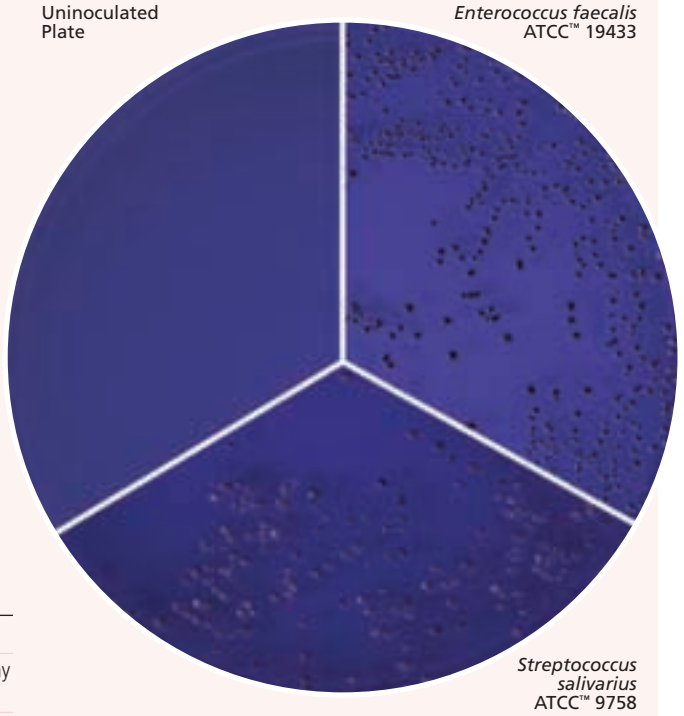
Appearance:	Colorless and clear to trace hazy.

Cultural Response

Difco™ Mitis Salivarius Agar with BBL™ Tellurite Solution 1%

Prepare the complete medium per label directions. Inoculate and incubate under 5-10% CO_2 at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	19433	10^2-10^3	Good	Blue/black
Escherichia coli	25922	10^3	Partial to complete inhibition	Brown, if any
Staphylococcus aureus	25923	10^3	Partial to complete inhibition	–
Streptococcus mitis	9895	10^2-10^3	Good	Blue
Streptococcus salivarius	9758	10^2-10^3	Good	Blue "gum drop" shape



Modified mTEC Agar

(See mTEC Agar, Modified)

Modified Thayer-Martin (MTM II) Agar

(See Thayer-Martin Selective Agar)

Moeller Decarboxylase Broths

(See Decarboxylase Differential Media)

Moeller KCN Broth Base

Intended Use

Moeller KCN Broth Base, when supplemented with a solution of potassium cyanide, is used in the differentiation of enteric bacilli on the basis of their ability to grow promptly in the presence of cyanide.

Summary and Explanation

In 1954, Moeller reported on the use of a medium containing cyanide as an aid in the differentiation of members of the *Enterobacteriaceae*.[1] Edwards and Ewing[2] modified Moeller Cyanide Broth and it is this modified formulation that is supplied as **BBL™** brand Moeller KCN Broth Base prepared in a tube.

This medium, when supplemented with potassium cyanide, is used in the differentiation of members of the genus *Salmonella* from *Citrobacter freundii*.[3] It is particularly useful in differentiating cultures of *C. freundii* that either fail to ferment lactose or ferment it slowly (i.e., strains formerly classified as Bethesda-Ballerup bacteria).[3] Except for strains of groups IV and V, members of the genus *Salmonella* do not grow in KCN medium, whereas species of *Citrobacter* with the exception of *C.* (*diversus*) *koseri* do grow in this medium.[3,4]

Principles of the Procedure
The addition of 0.15 mL of a 0.5% solution of potassium cyanide to each of the prepared tubes of the nutritive base enables differentiation of members of various genera within the *Enterobacteriaceae* family.

Procedure
Prior to use, add 0.15 mL of a 0.5% solution (0.5 g in 100 mL of *COLD* sterile distilled water) of potassium cyanide to each tube containing 10 mL of base and close tightly.

Precaution: Do not mouth pipette. Extreme care should be taken at all times in handling and disposing of potassium cyanide. Work should be performed within a chemical hood.

Inoculate Moeller KCN Broth with a loopful of an 18- to 24-hour culture of the test organism. Incubate at 35 ± 2°C in an aerobic atmosphere and examine daily for 2 days.

Expected Results
Except for a few infrequently-isolated species, members of the genera *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Morganella*, *Proteus* and *Providencia* are KCN positive; i.e., they grow in the presence of KCN. With certain exceptions, other *Enterobacteriaceae* are negative (inhibited) in the KCN test.[4]

References
1. Moeller. 1954. Acta Pathol. Microbiol. Scand. *34*:115.
2. Edwards and Ewing. 1955. Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis, Minn.
3. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
4. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability
BBL™ Moeller KCN Broth Base

BAM USDA

Cat. No. 221665 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*

Motility GI Medium

Intended Use
Motility GI Medium is used for detecting motility of microorganisms and for separating organisms in their motile phase.

Summary and Explanation
Motility GI Medium is prepared according to the formulation of Jordan, Caldwell and Reiter.[1] It is a semisolid gelatin-heart infusion medium that is adaptable to use in both tubes and plates for motility studies.

Principles of the Procedure
Beef heart infusion, peptone and gelatin provide nitrogen, vitamins and amino acids. Agar is the solidifying agent. Motility is evidenced by the presence of diffuse growth away from the line or spot of inoculation. Nonmotile organisms grow only along the line of inoculation.

Formula
Difco™ Motility GI Medium

Approximate Formula* Per Liter		
Beef Heart, Infusion from 500 g	10.0	g
Tryptose	10.0	g
Sodium Chloride	5.0	g
Gelatin	53.4	g
Agar	3.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 81.4 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
1. If tubes are desired, dispense the molten medium to a depth of 60-75 mm and cool in cold water up to the depth of the medium. Cool flasks of medium to 50-55°C; pour into sterile Petri dishes to a depth of 1/8 inch or more and allow to solidify.
2. Inoculate with growth from an 18-24 hour pure culture. If tubes are used, inoculate by stab inoculation. If plates are used, spot the inoculum on the surface or stab just below the medium surface.
3. Incubate at a temperature and duration appropriate for the suspected organism being tested.
4. Examine tubes or plates for growth and signs of motility.

Expected Results
Motility is evidenced by the presence of diffuse growth away from the line or spot of inoculation. Nonmotile organisms grow only along the line of inoculation.

Limitations of the Procedure
1. All weak or questionable motility test results should be confirmed by flagella stain or by direct wet microscopy.[2]
2. Some flagellar proteins are not synthesized at higher temperatures.[3]
3. Some isolates of *Yersinia enterocolitica* demonstrate motility at 35°C while others may be nonmotile at 25°C.[2] The motility of *Proteus* is also temperature dependent. This effect of temperature on motility needs to be taken into account when deciding on a testing regimen.
4. Due to the temperature dependency of motility in some organisms, a negative test tube or plate should be incubated an additional 5 days at a lower temperature of 22-25°C.[3]

User Quality Control

Identity Specifications

Difco™ Motility GI Medium

Dehydrated Appearance:	Light tan, free-flowing, slightly gritty, homogeneous.
Solution:	8.14% solution, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 8.14% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Motility GI Medium

Prepare the medium per label directions. Inoculate tubes of the medium with fresh cultures by stabbing with an inoculating wire and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	MOTILITY
Enterobacter aerogenes	13048	Good	+
Escherichia coli	25922	Good	+
Klebsiella pneumoniae	13883	Good	–
Proteus mirabilis	25933	Good	+/–*

**Motility of* Proteus *is temperature dependent, being more pronounced at 20°C and possibly absent at 35°C.*

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Klebsiella pneumoniae* ATCC™ 13883

References

1. Jordan, Caldwell and Reiter. 1934. J. Bacteriol. 27:165.
2. D'Amato and Tomfohrde. 1981. J. Clin. Microbiol. *14*:347.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Motility GI Medium

Cat. No. 286910 Dehydrated – 500 g

Motility Indole Lysine Sulfide (MILS) Medium

Intended Use

Motility Indole Lysine Sulfide (MILS) Medium is used to demonstrate motility, indole production, lysine decarboxylase and deaminase activity and hydrogen sulfide production for the differentiation of *Enterobacteriaceae*.

Summary and Explanation

MILS Medium is a modified formulation of Ederer et al. that combines a test for hydrogen sulfide production with tests for lysine decarboxylase and deaminase, indole production and motility.[1] When used in conjunction with Kligler Iron Agar or Triple Sugar Iron Agar and the urease test, it facilitates early detection and presumptive identification of enteric pathogens from feces and other clinical spcecimens.[2-4]

Principles of the Procedure

Enzymatic digests of gelatin, casein, animal tissue and heart infusion supply amino acids and other complex nitrogenous substances. Yeast extract is added to MILS Medium primarily to supply the B-complex vitamins. Dextrose is a source of energy.

A small amount of agar is added for demonstration of motility along a stab line of inoculation. Growth of motile organisms extends out from the line of inoculation, while nonmotile organisms grow only along the stab line.

The pH indicator bromcresol purple is used in MILS Medium to facilitate detection of decarboxylase activity. When inoculated with an organism that ferments dextrose, acids are produced that lower the pH, causing the indicator in the medium to change from purple to yellow.

The acidic pH also stimulates enzyme activity. Organisms that possess a specific decarboxylase degrade the amino acid provided in the medium, yielding a corresponding amine. Lysine decarboxylation yields cadaverine.

The production of these amines elevates the pH and causes the medium in the bottom portion of the tube to return to a purple color. The medium in the upper portion of the tube remains acidic because of the higher oxygen tension. If the organism being tested does not produce the required decarboxylase, the medium remains yellow (acidic) throughout or yellow with a purple or red reaction near the top.

Lysine deamination produces a color change in the upper portion of MILS Medium. Oxidative deamination of lysine yields a compound that reacts with ferric ammonium citrate, producing a burgundy red color in the top centrimeter of the medium.[3] (The bottom portion of the medium remains acidic.) This reaction can only be detected if lysine decaraboxylase is not produced, which is the case with *Proteus*, *Morganella* and *Providencia* species.

Indole is produced in MILS Medium by organisms that possess the enzyme tryptophanase. Tryptophanase degrades the typtophan present in the casein peptone, yielding indole. Indole can be detected in the medium by adding Kovacs' reagent to the agar surface. The indole combines with the *p*-dimethylaminobenzaldehyde of Kovacs' reagent and produces a red complex.

MILS Medium is also used in the demonstration of hydrogen sulfide production. Hydrogen sulfide, which is produced by some enteric organisms from sulfur compounds contained in the medium, reacts with ferric ion, producing a characteristic black precipitate.

Procedure

Using a sterile inoculating loop or needle, remove growth from the subculture medium and stab the center of the motility medium to the bottom of the tube. Incubate the tubes at 35°C for 48 hours.

Some flagellar proteins are not synthesized at higher temperatures. If motility tests are negative after two days incubation, place the cultures at 21-25°C for up to 5 days to induce flagellar development.

Expected Results

Motility is indicated by turbidity extending out from the line of stab inoculation. Nonmotile organisms grow only in the inoculated area.

Lysine decarboxylation (read at the bottom of the tube) is indicated by a dark purple color compared with an uninoculated control tube. In a negative test, the medium is yellow throughout the tube or has a narrow band of purple at the top of the medium. A negative test with organisms that do not ferment dextrose may show no color change.

The test for lysine deaminase is read in the upper portion of the medium. In a positive test, the medium at or near the surface is purple. In a negative test, the medium near the surface is yellow or remains unchanged.

Hydrogen sulfide production is indicated by blackening in the medium.

After reading the other reactions, indole production may be detected by adding three drops of Kovacs' reagent (Cat. No. 261185) to the surface of MILS Medium and shaking gently. The medium turns red if indole is present and remains unchanged if indole has not been produced.

References

1. Ederer, Lund, Blazevic, Reller and Mirrett. 1972. J. Clin. Microbiol. 2:266.
2. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
3. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Motility Indole Lysine Sulfide (MILS) Medium

Cat. No. 298266 Prepared Deeps (K Tubes), 5 mL – Ctn. of 100*

Difco™/BBL™ Indole Reagent

Cat. No. 261185 Droppers, 0.5 mL – Ctn. of 50

**Store at 2-8°C.*

Motility Nitrate (MN) Medium

Intended Use

Motility Nitrate (MN) Medium (Pickett's, for nonfermenters) is used for the detection of motility, nitrate reduction and denitrification of nonfermenting gram-negative bacilli.

Summary and Explanation

Motility Nitrate (MN) Medium is based on the formulation of Blackman and Pickett[1] and Pickett.[2] Microorganisms may be differentiated according to their metabolism of certain substrates. The ability to reduce nitrate to nitrite is characteristic of the family *Enterobacteriaceae*.[3] Nonfermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable of denitrification, which is a reduction of nitrate to nitrogen gas. The production of gas from nitrate is an important differential test for glucose-nonfermenting gram-negative bacilli. The end product of reduction depends upon the bacterial species.[4]

The medium is also used for indirect evidence of motility by nonfermenting gram-negative bacilli.

Principles of the Procedure

Enzymatic digests of casein and heart infusion supply amino acids and other complex nitrogenous substances. Yeast extract supplies the B-complex vitamins. Agar is added to demonstrate motility of the organism along a stab line of inoculation. Growth of motile organisms extends out from the line of inoculation.

Potassium nitrate is added to detect those nonfermenting gram-negative rods that can reduce nitrate to nitrite. Some organisms can further reduce the nitrite to nitrogen gas. Detection of denitrification activity can be useful for the identification of isolates, since only a small number of species of nonfermenting gram-negative rods can denitrify.[5-8]

Procedure

Loosen caps, place tubes in boiling water and cool to room temperature before use.

Organisms to be cultivated must first be isolated in pure culture on an appropriate solid medium.

Using a sterile straight, smooth inoculating needle, remove growth from the subculture medium and stab the center of the motility medium to 5-10 mm in depth. Incubate tubes at 18-20°C.[7]

Tubes may be examined for motility after 3-8 hours. If tubes are negative, reincubate and examine again after 24-48 hours. The opacity of the medium should be compared to an uninoculated tube.

To test for nitrate reduction (on cultures incubated 42-48 hours):

a. Add 0.5 mL of sulfanillic acid solution (Cat. No. 261197)
b. Add 0.5 mL of N, N-dimethly-1-naphthylamine solution (Cat. No. 261198).
c. Observe for the production of a pink to red color (positive nitrate reduction test). Since some organisms further reduce nitrite to ammonia, add a small amount of zinc dust (Cat. No. 261207) to tubes exhibiting no color.

Expected Results

Motility is indicated by turbidity extending out from the line of stab inoculation. Nonmotile organisms grow only in the inoculated area. After 3-8 hours of incubation, a small puffball of motility may be seen around the line of inoculation.[5] If this is not observed, tubes should be reincubated for 24-48 hours and compared for turbidity to an uninoculated tube. Negative motility reactions should be confirmed by a hanging drop preparation.

In the nitrate reduction test, a pink to red color develops after addition of the reagents if nitrite is present, and indicates that nitrate reduction has occurred. Since some organisms further reduce nitrite to ammonia, add a small amount of zinc dust (Cat. No. 261207) to tubes exhibiting no color. A pink color in this part of the test indicates no nitrate reduction. A colorless reaction indicates that nitrates have been completely reduced.

Consult appropriate references for an explanation of the reactions involved and expected results with specific microorganisms.[6-8]

References

1. Blachman and Pickett. 1978. Unusual aerobic bacilli in clinical bacteriology. Scientific Developments Press, Los Angeles, Calif.
2. Pickett. 1980. Nonfermentative gram-negative bacilli. Scientific Developments Press, Los Angeles, Calif.
3. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
4. MacFaddin. 2000. Biochemical tests for the identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
5. Gilardi (ed.). 1985. Nonfermentative gram-negative rods. Marcel Dekker, Inc., New York, N.Y.
6. Gilardi. 1988. Identification of glucose non-fermenting gram-negative rods. Dept. of Laboratories, North General Hospital, New York, N.Y.
7. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Motility Nitrate (MN) Medium

Cat. No. 296309 Prepared Tubes (K Tubes) – Pkg. of 10*

Difco™/BBL™ Nitrate A Reagent

Cat. No. 261197 Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate B Reagent

Cat. No. 261198 Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate C Reagent

Cat. No. 261207 Droppers, 1 g – Ctn. of 50

**Store at 2-8°C.*

Motility Test Medium

Intended Use

Motility Test Medium is used for the detection of motility of gram-negative enteric bacilli.

Summary and Explanation

In 1936, Tittsler and Sandholzer reported on the use of semisolid agar for the detection of bacterial motility.[1] Their original formulation has been modified in the medium supplied as BBL™ brand Motility Test Medium.

Principles of the Procedure

Bacterial motility can be observed directly from examination of the tubes following incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of nonmotile organisms only occurs along the stab line. TTC (triphenyltetrazolium chloride) may be added to facilitate the detection of motility. TTC is a redox indicator that is colorless in the oxidized form but becomes an insoluble red precipitate when reduced.

Formula

BBL™ Motility Test Medium

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Pancreatic Digest of Casein	10.0	g
Sodium Chloride	5.0	g
Agar	4.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 22 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. If desired, 5 mL of sterile 1% TTC solution may be added aseptically after autoclaving.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

BBL™ Motility Test Medium

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.2% solution, soluble in purified water upon boiling. Solution is pale to light, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Pale to light, yellow to tan, clear to slightly hazy.
Reaction of 2.2% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Motility Test Medium

Prepare the medium per label directions. Stab inoculate with fresh cultures and incubate at 35 ± 2°C for 2 days.

ORGANISM	ATCC™	RECOVERY	MOTILITY
Enterobacter aerogenes	13048	Good	+
Escherichia coli	25922	Good	+
Klebsiella pneumoniae	33495	Good	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	+
Shigella flexneri	9199	Good	–
Proteus vulgaris	8427	Good	+

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Klebsiella pneumoniae* ATCC™ 13883

Procedure

Inoculate tubes with a pure culture by stabbing the center of the column of medium to greater than half the depth. Incubate tubes for 24-48 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

After incubation, observe the tubes for growth in relation to the stab line. Nonmotile organisms grow only along the line of inoculation, while motile organisms spread out from the line of inoculation and may even grow throughout the medium.

Negative tubes can be reincubated at 25 ± 2°C for an additional 5 days, if desired.

Consult appropriate texts for results with specific organisms.[2,3]

Limitation of the Procedure

Many organisms fail to grow deep in semisolid media; inoculating pour plates may be advantageous.[4]

References

1. Tittsler and Sandholzer. 1936. J. Bacteriol. *31*:575.
2. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Motility Test Medium

BAM CCAM COMPF USDA

Cat. No.	211436	Dehydrated – 500 g
	221509	Prepared Tubes – Pkg. of 10
	221510	Prepared Tubes – Ctn. of 100

Difco™ TTC Solution 1%

Cat. No.	231121	Tube – 30 mL
	264310	Bottle – 25 g

Mucate Agar • Mucate Broth

Intended Use

These media are used in the differentiation of certain *Enterobacteriaceae* based on utilization of mucate.

Summary and Explanation

Kauffman and Peterson devised mucate medium (broth) for differentiation of some members of the *Enterobacteriaceae* based on their ability to utlize mucate as a source of carbon.[1] Mucate Agar is a solid form of mucate medium. Utilization of the mucate produces an acid reaction, which causes the medium to become yellow. Ellis et al. recommended using mucate medium and two other organic acid media (D-tartrate and sodium citrate) in conjunction with other tests for differentiation among *Salmonella* and *Arizona* strains.[2] Mucate utilization is also recommended for differentiation of other genera of the *Enterobacteriaceae*, such as *Escherichia coli* and *Shigella* species.[3-6]

Principles of the Procedure

Gelatin peptone provides amino acids and other nitrogenous substances to support bacterial growth.

Mucate, which is produced from mucic acid when the pH is adjusted with sodium hydroxide during manufacture of the medium, is the sole source of carbon. Utilization of the mucate results in an acid reaction, which lowers the pH of the medium and causes the bromthymol blue indicator to change the color of the medium from blue-green to yellow. The medium remains blue-green if the organism being tested does not utlize the mucate.

Mucate utilization is intended to be used in conjunction with other tests for differentiation and identification of certain members of the *Enterobacteriaceae*.

Procedure

Organisms to be tested must first be isolated in pure culture on an appropriate solid medium. Inoculate mucate media with a 3 mm loopful of a broth culture incubated overnight. Incubate mucate media at 35°C for up to 48 hours.

Expected Results

After sufficient incubation, examine tubes for a yellow (acid) reaction indicating utilization of the mucate. The medium remains blue-green if the mucate is not utilized. Consult appropriate texts for information needed to interpret the results.[3-5]

References

1. Kauffman and Petersen. 1956. Acta Pathol. Microbiol. Scand. *38*:481.
2. Ellis, Edwards and Fife. 1957. Pub. Health Lab. *15*:89.
3. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
4. Krieg and Holt (ed.). 1984. Bergey's manual of systematic bacteiology, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Mucate Agar

Cat. No. 297709 Prepared Slants (K Tubes) – Pkg. of 10*

BBL™ Mucate Broth

Cat. No. 296135 Prepared Tubes (K Tubes) – Pkg. of 10*

**Store at 2-8°C.*

Mueller Hinton Agars
Mueller Hinton Agar • Mueller Hinton II Agar
Mueller Hinton Agar with 5% Sheep Blood

Intended Use

Each lot of Mueller Hinton Agar and Mueller Hinton II Agar has been tested according to, and meets the acceptance limits of, the current M6 protocol published by the CLSI.

Mueller Hinton Agar is recommended for antimicrobial disc diffusion susceptibility testing of common, rapidly growing bacteria by the Bauer-Kirby method,[1-3] as standardized by the Clinical and Laboratory Standards Institute (CLSI).[4]

Mueller Hinton Agar with 5% Sheep Blood is recommended for antimicrobial disc diffusion susceptibility testing of *Streptococcus pneumoniae* with selected agents; i.e., chloramphenicol, erythromycin, ofloxacin, tetracycline and vancomycin, in addition to oxacillin screening for susceptibility to penicillin, as standardized by the Clinical and Laboratory Standards Institute (CLSI).[4]

NOTE: The recommended medium for disc diffusion susceptibility testing of *Streptococcus pneumoniae* is Mueller Hinton agar with 5% sheep blood. The recommended medium for *Haemophilus influenzae* is Haemophilus Test Medium (HTM) Agar. The recommended medium for *Neisseria gonorrhoeae* is GC Agar with 1% defined growth supplement (GC II Agar with **BBL™ IsoVitaleX™** Enrichment or equivalent). Interpretive criteria are provided in the CLSI Document M100 (M2),[5] which is included with CLSI Document M2, *Performance Standards for Antimicrobial Disk Susceptibility Tests*; Approved Standard.[4]

Summary and Explanation

Mueller Hinton Agar was originally developed for the cultivation of pathogenic *Neisseria*.[6] However, these organisms are now commonly isolated on selective media.

Because clinical microbiology laboratories in the early 1960s were using a wide variety of procedures for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents, Bauer, Kirby and others developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium.[1,2] A subsequent international collaborative study confirmed the value of Mueller Hinton Agar for this purpose because of the relatively good reproducibility of the medium, the simplicity of its formula, and the wealth of experimental data that had been accumulated using this medium.[7]

The CLSI has written a performance standard for the Bauer-Kirby procedure and this document should be consulted for additional details.[4] The procedure is recommended for testing rapidly growing aerobic or facultatively anaerobic bacterial pathogens, such as staphylococci, members of the *Enterobacteriaceae*, aerobic gram-negative rods; e.g., *Pseudomonas* spp. and *Acinetobacter* spp., enterococci and *Vibrio cholerae*. The procedure is modified for testing fastidious species; i.e., *H. influenzae*, *N. gonorrhoeae* and *S. pneumoniae* and other streptococci.

Mueller Hinton Agar and Mueller Hinton II Agar are manufactured to contain low levels of thymine and thymidine[8,9] and

User Quality Control

Identity Specifications

Difco™ Mueller Hinton Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 3.8% Solution at 25°C:	pH 7.3 ± 0.1

BBL™ Mueller Hinton II Agar

Dehydrated Appearance:	Fine, dry, homogeneous, free of extraneous material.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, trace hazy to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, trace hazy to slightly hazy.
Reaction of 3.8% Solution at 25°C:	pH 7.3 ± 0.1

Cultural Response

Difco™ Mueller Hinton Agar or BBL™ Mueller Hinton II Agar

Prepare the medium per label directions. Using the organisms listed below, inoculate plates, add antibiotic disks and incubate as recommended by CLSI.[4] Measure zone diameters and compare to the CLSI recommended zone ranges.[4]

ORGANISM	ATCC™
Enterococcus faecalis	33186
Escherichia coli	25922
Escherichia coli	35218
Pseudomonas aeruginosa	27853
Staphylococcus aureus	25923
Staphylococcus aureus	43300

controlled levels of calcium and magnesium.[10-12] Thymine and thymidine levels of raw materials are determined using the disc diffusion procedure with trimethoprim-sulfamethoxazole (SXT) discs and *Enterococcus faecalis* ATCC™ 33186 and/or 29212. Calcium and magnesium levels are controlled by testing raw materials and supplementing with sources of calcium and/or magnesium as required to produce correct zone diameters with aminoglycoside antibiotics and *Pseudomonas aeruginosa* ATCC 27853.[13]

Mueller Hinton agar complies with requirements of the World Health Organization[14] and is specified in the FDA *Bacteriological Analytical Manual* for food testing.[15]

Unsupplemented Mueller Hinton agar, although adequate for susceptibility testing of rapidly growing aerobic pathogens, is not adequate for more fastidious organisms such as *S. pneumoniae*. The CLSI Document M2, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, recommends Mueller Hinton agar supplemented with 5% defibrinated sheep blood. Details of quality control procedures and interpretive criteria for use with *S. pneumoniae* and other *Streptococcus* spp. are contained in supplemental tables.[5] These documents should be consulted for additional details.[4,5]

Principles of the Procedure

Acid hydrolysate (digest) of casein and beef extract supply amino acids and other nitrogenous substances, minerals, vitamins, carbon and other nutrients to support the growth of microorganisms. Starch acts as a protective colloid against toxic substances that may be present in the medium. Hydrolysis of the starch during autoclaving provides a small amount of dextrose, which is a source of energy. Agar is the solidifying agent.

The Bauer-Kirby procedure is based on the diffusion through an agar gel of antimicrobial substances which are impregnated on paper discs.[16] In contrast to earlier methods which used discs of high and low antimicrobial concentrations and which used the presence or absence of inhibition zones for their interpretation, this method employs discs with a single concentration of antimicrobial agent and zone diameters are correlated with minimal inhibitory concentrations (MIC).[1,2,4,7,16]

In the test procedure, a standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specified amounts of antibiotic or other antimicrobial agents are then placed on the surface of the medium, the plate is incubated and zones of inhibition around each disc are measured. The determination as to whether the organism is susceptible, intermediate or resistant to an agent is made by comparing zone sizes obtained to those in the CLSI Document M100(M2).[4]

Various factors have been identified as influencing disc diffusion susceptibility tests. These include the medium, excess surface moisture on the medium, agar depth, disc potency, inoculum concentration, pH and β-lactamase production by test organisms.[7,13,16]

Formulae

Difco™ Mueller Hinton Agar

Approximate Formula* Per Liter

Beef Extract Powder	2.0	g
Acid Digest of Casein	17.5	g
Starch	1.5	g
Agar	17.0	g

BBL™ Mueller Hinton II Agar

Approximate Formula* Per Liter

Beef Extract	2.0	g
Acid Hydrolysate of Casein	17.5	g
Starch	1.5	g
Agar	17.0	g

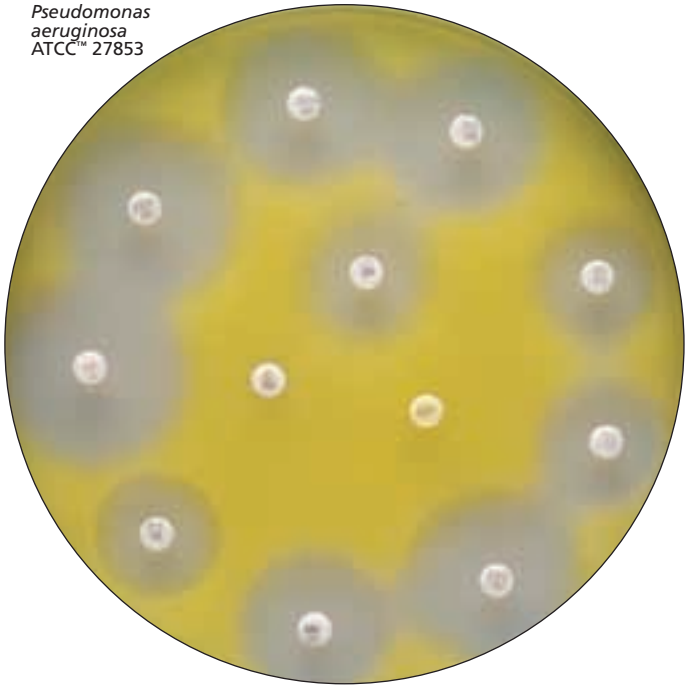
**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 38 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

Mueller Hinton II Agar

Pseudomonas aeruginosa ATCC™ 27853



Mueller Hinton Agar with 5% Sheep Blood

Streptococcus pneumoniae ATCC™ 49619

3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT. OPTIONAL: Cool medium to 45-50°C and aseptically add 5% sterile defibrinated sheep blood.
4. Pour cooled Mueller Hinton agar into sterile Petri dishes on a level, horizontal surface to give a uniform depth of about 4 mm (60-70 mL of medium for 150 mm plates and 25-30 mL for 100 mm plates) and cool to room temperature.[4]
5. Check prepared medium to ensure the final pH is 7.3 ± 0.1 at 25°C.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

A. Standard Method[4]

1. Perform a Gram stain before starting a susceptibility test to confirm culture purity and to determine appropriate test battery.
2. Select at least three to five well-isolated similar colonies and transfer with an inoculation needle or loop into 4-5 mL of suitable broth.
3. Incubate the broth at 35°C until it achieves or just exceeds the turbidity of the 0.5 McFarland barium sulfate standard (usually 2-6 hours). This results in a suspension containing approximately 1 to 2 × 10^8 CFU/mL (for *E. coli* ATCC 25922).
4. Adjust the turbidity to be equivalent to the barium sulfate standard. For the diluent, use sterile broth or sterile saline. The turbidity of the standard and the test inoculum should be compared by holding both tubes in front of a white background with finely drawn black lines or a photometric device can be used.
5. Within 15 minutes after adjusting the turbidity of the inoculum, immerse a sterile cotton swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
6. Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streakings to obtain even inoculation. As a final step, swab the rim of the agar bed.
7. The lid may be left ajar for 3-5 minutes and the plate held at room temperature for no longer than 15 minutes to allow any surface moisture to be absorbed before applying the antimicrobial agent-impregnated discs.
8. Apply the discs by means of an antimicrobial disc dispenser, using aseptic precautions. Deposit discs so that the centers are at least 24 mm apart. It is preferable to deposit penicillin and cephalosporin discs so that they are not less than 10 mm from the edge of the Petri dish, and their centers are at least 30 mm apart. **Avoid placing such discs adjacent to one another.** After discs have been placed on the agar, tamp them with a sterile needle or forceps to make complete contact with the medium surface. This step is not necessary if the discs are deposited using the **Sensi-Disc**™ 12-place self-tamping dispenser.
9. Within 15 minutes after the discs are applied, invert the plates and place them in a 35°C incubator. *With non-fastidious organisms, plates should not be incubated under an increased concentration of carbon dioxide.*
10. Examine plates after 16-18 hours incubation. A full 24 hours incubation is recommended for *Staphylococcus aureus* with oxacillin to detect methicillin-resistant *S. aureus* (MRSA) and for *Enterococcus* spp. when tested with vancomycin to detect vancomycin-resistant strains. Growth within the apparent zone of inhibition is indicative of resistance.

A confluent "lawn" of growth should be obtained. If only isolated colonies grow, the inoculum was too light and the

test should be repeated. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using sliding calipers, a ruler, or a template prepared for this purpose. The measuring device is held on the back of the inverted plate over a black, non-reflecting background, and illuminated from above.

The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies which can be detected with difficulty near the edge of the obvious zone of inhibition. *Staphylococcus aureus* when tested with oxacillin discs is an exception, as are enterococci when tested with vancomycin. In these cases, transmitted light should be used to detect a haze of growth around the disc which is shown by "occult resistant" MRSA strains[17] or vancomycin-resistant enterococci.[4] With *Proteus* species, if the zone of inhibition is distinct enough to measure, disregard any swarming inside the zone. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

B. Direct Method[4]

The direct colony suspension method should be used when testing *S. pneumoniae*. Observe aseptic techniques.

1. Suspend growth from an overnight (16-18 hour) sheep blood agar plate in saline or broth, such as Mueller Hinton broth. Adjust the turbidity to be equivalent to the 0.5 McFarland barium sulfate standard. For the diluent, use sterile broth or sterile saline. The turbidity of the standard and the test inoculum should be compared by holding both tubes in front of a white background with finely drawn black lines or a photometric device can be used.
   NOTE: Alternative methods of inoculum preparation involving devices that permit direct standardization of inocula without adjustment of turbidity, such as the **BBL™ Prompt™** Inoculation System, have been found to be acceptable for routine testing purposes.[18]
2. Within 15 minutes of adjusting the turbidity of the inoculum, dip a sterile swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
3. Inoculate onto Mueller Hinton Agar with 5% Sheep Blood by streaking the entire agar surface of the plate three times, rotating the plate 60° between streakings to obtain even inoculation. As a final step, swab the rim of the agar bed.
4. Replace the lid of the plate and hold the plate at room temperature for at least 3 minutes, but no longer than 15 minutes, to allow surface moisture to be absorbed before applying the drug-impregnated discs. Use no more than nine discs per 150 mm plate, or four discs per 100 mm plate.
5. Incubate for 20-24 hours at 35°C in an atmosphere of 5% CO_2.

Expected Results

Zone diameters measured around discs should be compared with those in the CLSI Document M100 (M2). Results obtained with specific organisms may then be reported as resistant, intermediate or susceptible.

With Mueller Hinton Agar with 5% Sheep Blood, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. The zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. Zone diameters for the agents specified under "Intended Use" should be compared with those in the CLSI Document M100 (M2), which provides interpretive criteria.[5] Results obtained may then be reported as resistant, intermediate or susceptible.

Isolates of *S. pneumoniae* with oxacillin zone diameters of ≥20 mm are susceptible (MIC ≤0.06 mg/mL) to penicillin. CLSI Document M100 (M2) should be consulted for other antimicrobial agents to which penicillin-susceptible isolates may also be considered susceptible.[4]

NOTE: Informational supplements to CLSI Document M2, containing revised tables of antimicrobial discs and interpretive standards are published periodically. The latest tables should be consulted for current recommendations. The complete standard and informational supplements can be ordered from the Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898. Telephone: (610) 688-1100.

Refer to other texts for additional information on antimicrobial susceptibility testing.[19,20] Protocols developed by the CLSI and used by manufacturers to evaluate the performance of Mueller Hinton Agar in comparison to a reference medium are published in CLSI document M6-A.[21]

Limitations of the Procedure

1. Numerous factors can affect results: inoculum size; rate of growth; medium formulation and pH, length of incubation and incubation environment; disc content and drug diffusion rate; and measurement of endpoints. Therefore, strict adherence to protocol is required to ensure reliable results.[22]
2. When Mueller Hinton agar is supplemented with blood, the zone of inhibition for oxacillin and methicillin may be 2-3 mm smaller than those obtained with unsupplemented agar.[23] Conversely, sheep blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci.[24] Sheep blood may cause indistinct zones or a film of growth within the zones of inhibition around sulfonamide and trimethoprim discs.[23]
3. Mueller Hinton agar deeper than 4 mm may cause false-resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility report.[23]
4. A pH outside the range of 7.3 ± 0.1 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excessive activity.[23] The opposite effects are possible if the pH is too high.[23]

References

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2. Ryan, Schoenknecht and Kirby. 1970. Hospital Practice *5*:91.
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15. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
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17. Hindler and Anderbied. 1985. J. Clin. Microbiol. *21*:205.
18. Baker, Thornsberry and Hawkinson. 1983. J. Clin. Microbiol. *17*:450.
19. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.
20. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
21. Clinical and Laboratory Standards Institute. 2006. Approved standard: M6-A2. Protocols for evaluating dehydrated Mueller-Hinton agar, 2nd ed. CLSI, Wayne, Pa.
22. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
23. Wood and Washington. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
24. Buschelman, Jones and Bale. 1994. J. Clin. Microbiol. *32*:565.

Availability

Difco™ Mueller Hinton Agar

BAM BS12 CCAM CLSI CMPH2 ISO MCM9

Cat. No.	225250	Dehydrated – 500 g
	225220	Dehydrated – 2 kg
	225230	Dehydrated – 10 kg

BBL™ Mueller Hinton II Agar

BAM BS12 CCAM CLSI CMPH2 ISO MCM9

Cat. No.	211438	Dehydrated – 500 g
	211441	Dehydrated – 5 lb (2.3 kg)
	212257	Dehydrated – 25 lb (11.3 kg)

United States and Canada

Cat. No.	221177	Prepared Plates – Pkg. of 20*
	221275	Prepared Plates – Ctn. of 100*
	221994	Prepared Plates (150 × 15 mm) – Pkg. of 8*
	221800	Prepared Plates (150 × 15 mm) – Box of 24*

Europe

Cat. No.	254032	Prepared Plates – Pkg. of 20*
	254081	Prepared Plates – Ctn. of 120*
	254062	Prepared Plates (150 × 15 mm-style) – Pkg. of 20*
	254518	Prepared Plates (square 120 × 120 mm-style) – Pkg. of 20*

Japan

Cat. No.	251177	Prepared Plates – Pkg. of 20*
	251275	Prepared Plates – Ctn. of 100*
	252128	Prepared Plates – Ctn. of 100 (× 2)*
	251579	Prepared Plates – Ctn. of 200*
	251800	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*

Mexico

Cat. No.	252624	Prepared Plates – Pkg. of 10*

BBL™ Mueller Hinton Agar with 5% Sheep Blood

BS12 CCAM CLSI CMPH2 MCM9

United States and Canada

Cat. No.	221176	Prepared Plates – Pkg. of 20*
	221993	Prepared Plates (150 × 15 mm) – Pkg. of 8*
	221801	Prepared Plates (150 × 15 mm) – Box of 24*

Europe

Cat. No.	254030	Prepared Plates – Pkg. of 20*
	254080	Prepared Plates – Ctn. of 120*
	255080	Prepared Plates (150 × 15 mm-style) – Pkg. of 20*
	254517	Prepared Plates (square 120 × 120 mm-style) – Pkg. of 20*

Japan

Cat. No.	251176	Prepared Plates – Pkg. of 20*
	252129	Prepared Plates – Ctn. of 200*
	251801	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*

**Store at 2-8°C.*

Mueller Hinton Chocolate Agar

Intended Use

Mueller Hinton Chocolate Agar is for use in qualitative procedures for the isolation and cultivation of fastidious organisms, particularly *Haemophilus* species. It was formerly recommended by the Clinical and Laboratory Standards Institute (CLSI) for antimicrobial susceptibility testing of *H. influenzae*. However, it has been replaced in this procedure by Haemophilus Test Medium (HTM) Agar.

Summary and Explanation

Mueller Hinton Agar was originally developed for the cultivation of pathogenic *Neisseria*.[1] In the 1960s, Bauer, Kirby and others developed a standardized disc diffusion procedure for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents in which Mueller Hinton Agar was selected as the test medium.[2]

Because growth of fastidious organisms was poor, the use of Mueller Hinton Agar supplemented with 1% hemoglobin and a defined supplement was adopted for testing of *H. influenzae*.[3] Known as Mueller Hinton Chocolate Agar, this formulation was replaced for this purpose by HTM Agar.[4]

The medium is now recommended for routine cultivation of fastidious organisms.

Principles of the Procedure

The primary nutrients in Mueller Hinton Chocolate Agar are beef extract, which provides nitrogenous nutrients, vitamins and minerals required for microbial growth, and acid hydrolysate of casein, which provides amino acids with the exception of cystine (because casein contains little cystine) and tryptophan (which is destroyed by the acid treatment, as are vitamins). The starch neutralizes toxic fatty acids that may be present in the agar. Hemoglobin provides X factor (hemin) for *Haemophilus*

species. **BBL™ IsoVitaleX™** Enrichment is a defined supplement that provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, co-enzymes, dextrose, ferric ion and other factors for improved growth of fastidious organisms; i.e., pathogenic *Neisseria*.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 35 ± 2°C for 18-24 hours and up to 72 hours, if necessary, in an aerobic atmosphere enriched with 5-10% CO_2.[5]

Expected Results

After a minimum of 18 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

The growth of *Haemophilus* appears as small (1 mm), moist, pearly colonies with a characteristic "mousy" odor.

References

1. Mueller and Hinton. 1941. Proc. Soc. Exp. Biol. Med. *48*:330.
2. Bauer, Kirby, Sherris and Turck. 1966. Am. J. Clin. Pathol. *45*:493.
3. National Committee for Clinical Laboratory Standards. 1984. Approved standard: M2-A3. Performance standards for antimicrobial disk susceptibility tests, 3rd ed. NCCLS, Villanova, Pa.
4. National Committee for Clinical Laboratory Standards. 1990. Approved standard: M2-A4. Performance standards for antimicrobial disk susceptibility tests, 4th ed. NCCLS, Villanova, Pa.
5. Campos. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Mueller Hinton Chocolate Agar

United States and Canada

Cat. No.	221860	Prepared Plates – Pkg. of 20*
	221869	Prepared Plates (150 × 15 mm-style plates) – Pkg. of 8*
	221802	Prepared Plates (150 × 15 mm-style plates) – Box of 24*

Japan

Cat. No.	251860	Prepared Plates – Pkg. of 20*
	251802	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*

**Store at 2-8°C.*

Mueller Hinton II Agar with Tryptophan

Intended Use

Mueller Hinton II Agar with tryptophan is for the determination of indole production.

Summary and Explanation

The ability of an organism to produce indole has long been used as a part of the IMVIC reactions to separate *Escherichia coli* from *Klebsiella-Enterobacter* organisms, although it is now considered important in the identification of a wide variety of organisms isolated in the clinical laboratory.[1]

Principles of the Procedure

Indole is produced by bacteria through degradation of the amino acid, tryptophan. Various enzymes involved are collectively called "tryptophanase," a general term used to denote the complete system of enzymes that mediate the production of indole by hydrolytic activity against the substrate tryptophan.[2]

The indole production by the organisms can be detected with Kovacs' Reagent (Cat. No. 261185). Indole, if present, combines with the aldehyde present in the reagent to give a red color.

Procedure

Inoculate the medium with a pure culture, streaking to obtain isolated colonies. Place a sterile blank disc on the inoculated plate. Incubate plates at 35 ± 2°C in an aerobic atmosphere for 18-24 hours.

After incubation, add a few drops of Kovacs' Reagent to the sterile blank disc. Read the disc for the indole reaction within 1 minute after the addition of the reagent.

Expected Results

The production of indole is indicated by a brown red or purple red color which develops on the disc within 1 minute. If no indole is produced, no color will develop with the reagent; the original color of the reagent is a negative test.

References

1. Blazevic and Ederer. 1975. Principles of biochemical tests in diagnostic microbiology. John Wiley & Sons, New York, N.Y.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Mueller Hinton II Agar with Tryptophan

Cat. No.	297623	Prepared Plates (150 × 15 mm-style plates) – Box of 24*

Difco™/BBL™ Indole Reagent

Cat. No.	261185	Droppers, 0.5 mL – Ctn. of 50

**Store at 2-8°C.*

Mueller Hinton Broth (Not Cation-Adjusted)

Intended Use

Mueller Hinton Broth is a general purpose medium that may be used in the cultivation of a wide variety of fastidious and nonfastidious microorganisms. This medium is not supplemented with calcium or magnesium ions.

Summary and Explanation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria*.[1] Other media were developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria*, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. It is now used as a test medium for antimicrobial susceptibility testing.[2]

Mueller Hinton Broth, not cation-adjusted, has a formula similar to that of the solid medium, but without agar, for use when the fluid medium is preferred. While it may be used for the general cultivation of bacteria, for consistency, cation-adjusted Mueller Hinton Broth is now recommended for dilution antimicrobial susceptibility testing of all species of most commonly encountered aerobic and facultatively anaerobic bacteria.[2,3] **BBL**™ Mueller Hinton II Broth is cation-adjusted to the calcium and magnesium ion concentrations recommended in the CLSI standard M7.[2]

Difco™ Mueller Hinton Broth, not cation-adjusted, is formulated to have a low thymine and thymidine content. It may be used for broth dilution antimicrobial susceptibility testing as long as the calcium and magnesium ion concentrations are adjusted according to CLSI standard M7.[2]

BBL™ Mueller Hinton Broth, not cation-adjusted, has not been formulated to have a low thymine and thymidine content. It may be used for the general cultivation of bacteria.

Principles of the Procedure

Acid hydrolysate (digest) of casein and beef extract supply amino acids and other nitrogenous substances, minerals, vitamins, carbon and other nutrients to support the growth of microorganisms. Starch acts as a protective colloid against toxic substances that may be present in the medium. Hydrolysis of the starch during autoclaving provides a small amount of dextrose, which is a source of energy.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Mueller Hinton Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous with a few dark specks.
Solution:	2.1% solution, soluble in purified water upon boiling. Solution is very light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear, may have a slight precipitate.
Reaction of 2.1% Solution at 25°C:	pH 7.3 ± 0.1
Calcium:	2.9-5.9 mg/L
Magnesium:	3.2-5.2 mg/L

Cultural Response

Difco™ Mueller Hinton Broth

Prepare the medium per label directions, supplementing with calcium and magnesium ions according to CLSI standard M7.[2] Prepare broth microdilution trays, inoculate (with the organisms listed below) and incubate as recommended by CLSI.[2] Compare the MIC (lowest concentration of antimicrobial that inhibits growth of the test bacterium) of the antimicrobials tested to the CLSI standard.[2]

ORGANISM	ATCC™
Enterococcus faecalis	29212
Escherichia coli	25922
Pseudomonas aeruginosa	27853
Staphylococcus aureus	29213

Identity Specifications

BBL™ Mueller Hinton Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.2% solution, soluble in purified water upon boiling. Solution is pale to light, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Pale to light, tan to yellow, clear to slightly hazy.
Reaction of 2.2% Solution at 25°C:	pH 7.3 ± 0.1

Cultural Response

BBL™ Mueller Hinton Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (up to 72 hours, if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	≤10^3	Good
Enterococcus faecalis	33186	≤10^3	Good
Escherichia coli	25922	≤10^3	Good
Pseudomonas aeruginosa	27853	≤10^3	Good
Staphylococcus aureus	29213	≤10^3	Good

Formulae

Difco™ Mueller Hinton Broth

Approximate Formula* Per Liter		
Beef Extract Powder	2.0	g
Acid Digest of Casein	17.5	g
Starch	1.5	g

BBL™ Mueller Hinton Broth

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Acid Hydrolysate of Casein	17.5	g
Starch	1.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Mueller Hinton Broth – 21 g;
   BBL™ Mueller Hinton Broth – 22 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 116-121°C for 10-15 minutes (consult product label). DO NOT OVERHEAT.
4. Check prepared medium to ensure the final pH is 7.3 ± 0.1 at 25°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on broth dilution antimicrobial susceptibility testing, refer to the appropriate procedures outlined in the references.[2-5]

Organisms to be subcultured must first be isolated in pure culture on an appropriate solid medium. Transfer growth from the isolation medium to Mueller Hinton Broth using standard bacteriologic techniques.[3,4]

For enrichment purposes, inoculate the specimen onto primary media and then into the broth, according to recommended procedures.

Incubate the tubes at 35°C under conditions appropriate for the organism being cultured.

Expected Results

For broth dilution antimicrobial susceptibility testing, refer to appropriate references for results.[2,5]

Growth in broth media is indicated by the presence of turbidity compared with an uninoculated control.

References

1. Mueller and Hinton. 1941. Proc. Soc. Exp. Biol. Med. *48*:330.
2. Clinical and Laboratory Standards Institute. 2006. Approved Standard: M7-A7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. CLSI, Wayne, Pa.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
5. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington , D.C.

Availability

Difco™ Mueller Hinton Broth (Not cation-adjusted)

CLSI

Cat. No.	275730	Dehydrated – 500 g
	275710	Dehydrated – 2 kg

BBL™ Mueller Hinton Broth (Not cation-adjusted)

Cat. No.	211443	Dehydrated – 500 g
	296195	Prepared Tubes, 2 mL (K Tubes) – Pkg. of 10
	296164	Prepared Tubes, 2 mL (K Tubes) – Ctn. of 100
	297220	Prepared Tubes, 5 mL (C Tubes) – Pkg. of 10
	295834	Prepared Tubes, 5 mL (C Tubes) – Ctn. of 100
Europe		
Cat. No.	257092	Prepared Bottles, 900 mL – Pkg. of 4

Mueller Hinton II Broth (Cation-Adjusted) Mueller Hinton II Broth (Cation-Adjusted) with 2% Sodium Chloride

Intended Use

Mueller Hinton II Broth is intended for use in quantitative procedures for susceptibility testing of rapidly-growing aerobic and facultatively anaerobic bacteria isolated from clinical specimens. It is formulated to have a low thymine and thymidine content and is adjusted to the calcium and magnesium ion concentrations recommended in CLSI standard M7.[1]

Mueller Hinton II Broth with 2% Sodium Chloride (NaCl) is for testing methicillin-resistant strains of *Staphylococcus aureus* (MRSA).[1]

Summary and Explanation

The development of laboratory tests to determine the activity of antimicrobial agents has paralleled the development of these agents. In 1929, Fleming used a serial dilution technique to measure the lowest concentration of penicillin that prevented growth of a test organism in broth.[2] Ericsson and Sherris published an excellent review of the various methods for susceptibility testing and the relationship of dilution and diffusion methods.[3]

Rammelkamp and Maxon were among the earliest to use the tube dilution test to determine the *in vitro* antimicrobial

susceptibility of bacteria isolated from clinical specimens.[4] The development of this test resulted from the need to know why some patients infected with *S. aureus* did not respond to penicillin therapy.

The tube dilution test (broth dilution) involves exposing bacteria to decreasing concentrations of antimicrobial agents in liquid media, usually by serial two-fold dilution. The mixture, consisting of microorganisms, nutrient medium and antimicrobial agent, is incubated at 35°C for 16-20 hours. The lowest concentration of antimicrobial agent at which no visible growth occurs is defined as the minimal inhibitory concentration (MIC).

The term "microdilution" appeared in the literature in 1970 to describe the minimal inhibitory concentration tests performed with volumes of 0.1 mL or less of antimicrobial solution.[5] Correlations between MIC values using microdilution and tube dilution methodologies have been reported to be between 85 and 96%.[6,7]

The qualitative disc diffusion antimicrobial susceptibility procedure has been standardized since 1966.[8] The rationale for an MIC susceptibility test rather than the disc diffusion test is that it gives quantitative information. It provides a relationship between the amount of antimicrobial agent required to inhibit the growth of an organism *in vitro* and the achievable concentrations in the blood, urine, cerebrospinal fluid or bile, under various dosage conditions. It has been suggested that in the treatment of systemic infections, the drug dosage should yield a peak concentration at the site of infection that is two to four times greater than the MIC value, while for urinary tract infections, a peak urine concentration of 10-20 times the MIC value should be achieved.[9] However, effective antimicrobial therapy also depends on many other factors.[10]

Cation-adjusted Mueller Hinton Broth is the medium usually used for dilution antimicrobial susceptibility tests. This medium is supplemented with calcium and magnesium salts to produce correct MICs with aminoglycosides and *Pseudomonas aeruginosa*.[1] However, this medium is not satisfactory for fastidious organisms such as *S. pneumoniae*. Cation-adjusted Mueller Hinton Broth supplemented with 2-5% lysed horse blood is the medium recommended for susceptibility testing of *S. pneumoniae*.[1]

Thornsberry and McDougal reported that adding 2% sodium chloride to cation-adjusted Mueller Hinton Broth improved the reliability of MIC tests using oxacillin for detecting methicillin-resistant *S. aureus* (MRSA).[11] In addition, they recommend the alternative direct inoculum standardization procedure (see "Procedure," step 2) and incubation of the inoculated MIC trays or tubes for a full 24 hours.[11]

User Quality Control

Identity Specifications

BBL™ Mueller Hinton II Broth (Cation-Adjusted)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.2% solution, soluble in purified water upon boiling. Solution is pale to light yellow to tan, clear to slightly hazy.
Prepared Appearance:	Pale to light yellow to tan, clear to slightly hazy.
Reaction of 2.2% Solution at 25°C:	pH 7.3 ± 0.1
Calcium:	20-25 mg/L
Magnesium:	10-12.5 mg/L

Cultural Response

BBL™ Mueller Hinton II Broth (Cation-Adjusted)

Prepare the medium per label directions. Inoculate with approximately 10^5 of the test organisms, dispense into an antimicrobial susceptibility test system and incubate at 35 ± 2°C for 16-20 hours.

ORGANISM	ATCC™	RESULT
Enterococcus faecalis	29212	Satisfactory MIC values
Escherichia coli	25922	Satisfactory MIC values
Pseudomonas aeruginosa	27853	Satisfactory MIC values
Staphylococcus aureus	29213	Satisfactory MIC values

Principles of the Procedure

Acid hydrolysate of casein and beef extract provide nutrients for growth of test organisms. These ingredients are selected for low thymine and thymidine content as determined by MIC values with *Enterococcus faecalis* and sulfamethoxazole-trimethoprim (SXT). Calcium and magnesium ion concentrations are adjusted to provide the amounts recommended by CLSI to give the correct MIC values with aminoglycosides and *P. aeruginosa*.[1] The pH has been adjusted to the specification in CLSI standard M7.

MRSA cultures often consist of two populations, one that is susceptible and one that is resistant (so-called "occult resistant" strains). The methicillin-resistant population grows more slowly and prefers a high salt concentration as contained in Mueller Hinton II Broth with 2% NaCl. In addition, the lower pH of this medium (6.9) improves the stability of β-lactam antibiotics during storage of prepared MIC test tubes or trays.[12]

Antimicrobial agents are prepared in serial two-fold dilutions in Mueller Hinton II Broth and are inoculated with the test culture to give a final concentration of 5×10^5 CFU/mL. Following incubation at 35°C, the presence of turbidity indicates growth of the organism. The lowest concentration of antimicrobial agent showing no growth is the MIC of that organism for that agent. The interpretation as to whether the organism is susceptible, intermediate, or resistant in its response to the agent is made by comparing the MIC to those in the MIC interpretive standards in CLSI standard M7.[1,13]

Various factors have been identified as influencing broth dilution susceptibility tests. These include the medium, antimicrobial potency, inoculum concentration, pH, antimicrobial stability and mechanisms of resistance by the test organisms.[3,14,15]

Formula

BBL™ Mueller Hinton II Broth (Cation-Adjusted)

Approximate Formula* Per Liter

Beef Extract	3.0	g
Acid Hydrolysate of Casein	17.5	g
Starch	1.5	g

**Adjusted and/or supplemented as required with appropriate salts to provide 20-25 mg/L of calcium and 10-12.5 mg/L of magnesium and as additionally required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 22 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 116-121°C for 10 minutes. DO NOT OVERHEAT.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Mueller Hinton II Broth (Cation-Adjusted) may be used for inoculum preparation for MIC tests and for preparation of antimicrobial dilutions for the microdilution or macrodilution procedure. Details for the preparation of antimicrobial agents are provided in reference 1.

1. Inoculum Standardization (for rapidly growing bacteria)
   a. Using aseptic technique, pick 3-5 isolated colonies of the same organism from an 18- to 24-hour **Trypticase**™ Soy Agar with 5% Sheep Blood (TSA II) plate and inoculate into 5 mL of Mueller Hinton II Broth.
   b. Incubate 2-6 hours at 35°C. Periodically check turbidity against the 0.5 McFarland turbidity standard.
      - If comparable, go to step 3, Inoculation of Antimicrobial Dilutions.
      - If too turbid, dilute aseptically with additional Mueller Hinton II Broth and repeat turbidity check. If turbidity is comparable to the standard, go to step 3, Inoculation of Antimicrobial Dilutions.
      - If not turbid enough, continue incubation. When turbidity is comparable to the standard, go to step 3, Inoculation of Antimicrobial Dilutions.

   Suspensions of test organisms must be used within 15 minutes of standardization.
2. Alternative Direct Inoculum Standardization (for rapidly growing bacteria and MRSA)
   A stationary phase culture may also be used. In this method, skip step number 1b and simply suspend enough colonies in the broth to equal the turbidity of the 0.5 McFarland standard. For MRSA, use Mueller Hinton II Broth with 2% NaCl. Suspensions of test organisms must be used within 15 minutes of standardization.
3. Inoculation of Antimicrobial Dilutions
   The amount of inoculum depends on the procedure used.[1] The standardized inoculum prepared above will contain approximately 1-2 × 10^8 CFU/mL. The final concentration in a well (or tube) should be 5 × 10^5 CFU/mL (*not* CFU/tube or well).
   a. Macrodilution (tube) method
      If the volume of antimicrobial solution in the tube is 1 mL, dilute the standardized inoculum 1:100 in Mueller Hinton II Broth (0.1 mL to a 10-mL tube of broth). Add 1.0 mL of the adjusted inoculum to each tube containing an antimicrobial agent and 2.0 mL to a sterile empty tube for a growth control.
   b. Microdilution method
      In this method, the antimicrobial dilutions are made in sterile plastic trays with round or conical-shaped wells. The volume is either 0.05 or 0.1 mL in each well. If the volume in the well is 0.1 mL, dilute the inoculum 1:10 and add 0.005 mL of the inoculum per well, using a replicator. One well in each tray should contain 0.1 mL of broth without any antimicrobial agent (growth control well).
      If a dropper (0.05 mL) is used for the inoculum and the volume of antimicrobial solution is 0.05 mL, this results in a 1:2 dilution. Therefore, dilute the inoculum 1:100 and add 0.05 mL to each well to obtain the final concentration of 5 × 10^5 CFU/mL (5 × 10^4 CFU/well). Add 0.05 mL of broth without any antimicrobial agent (growth control well). After the trays are inoculated, cover with tape or a tight-fitting lid to prevent evaporation.
4. Incubation
   Incubate the tubes or trays (stacked no more than four high) at 35°C for 16-20 hours for Mueller Hinton II Broth and a full 24 hours for Mueller Hinton II Broth with 2% Sodium Chloride (MRSA). Do not use a CO_2 incubator. To prevent drying out, the trays should be covered with plastic tape, a tight fitting lid, or placed in a plastic bag.[1]
   Control cultures should be included each time a susceptibility test is performed or weekly if satisfactory performance can be documented according to the CLSI standard.[1] The correct quality control MIC ranges will be found in M100 (M7).

Expected Results

The minimal inhibitory concentration (MIC) of an antimicrobial agent for a specific organism is the lowest concentration which will inhibit the growth of the organism. Growth is indicated by turbidity or sediment. Some microorganisms when tested against trimethoprim/sulfamethoxazole or sulfonamides alone do not always give clear-cut end points. In the case of doubling dilutions of trimethoprim/sulfamethoxazole, there may be a "trailing" of growth. Such a pattern typically shows an obvious reduction in the amount of growth and, then, either small pellets (usually less than 1 mm in diameter) in the rest of the wells, or an obvious reduction in the amount of growth and then a slight but detectable graduation in the size of the pellets. In these cases, the MIC end point should be identified as the lowest concentration of antimicrobial agent beyond which there is no further reduction in the size of the pellet or amount of turbidity.

An organism may be susceptible, intermediate or resistant for a given antimicrobial agent depending on the MIC value. Interpretive standards for MIC values with various drugs may be found in CLSI document M100 (M7)[1] or may be obtained from the drug manufacturer.

NOTE: Informational supplements to CLSI Document M7, containing revised tables of antimicrobial agents and interpretive standards are published periodically. The latest tables should be consulted for current recommendations. The complete standard and informational supplements can be ordered from the Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898. Telephone: (610) 688-1100.

Refer to other texts for additional information on antimicrobial susceptibility testing.[16,17]

References

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16. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.
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Availability

BBL™ Mueller Hinton II Broth (Cation-Adjusted)

BS12 CLSI CMPH2 MCM9

Cat. No.	212322	Dehydrated – 500 g
	297701	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10
	298268	Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100
	297310	Prepared Bottle (pH 7.3) – 250 mL
	297963	Prepared Bottle – 400 mL

BBL™ Mueller Hinton II Broth (Cation-Adjusted) with 2% Sodium Chloride

CLSI

Cat. No.	297311	Prepared Bottle (pH 6.9) – 250 mL

Muller Kauffmann Tetrathionate Broth Base

Intended Use

Muller Kauffmann Tetrathionate Broth Base is used for enriching *Salmonella* from food and environmental samples prior to selective isolation.

Summary and Explanation

Salmonellosis is one of the most important and most frequently reported human foodborne diseases worldwide.[1] Outbreaks have been associated with the consumption of pork and pork products,[2,3] broiler chickens,[4] and other animals. Environmental sources include animal feed, litter and dust from hen houses, and animal feces.

The process of isolating *Salmonella* from food is often difficult. The key to successful recovery lies in obtaining the contaminated portion of test sample. Even when the contaminated material has been obtained, *Salmonella* may be present only in small numbers and accompanied by larger numbers of other contaminating bacteria. Pre-enrichment is necessary to permit the detection of low numbers of *Salmonella* or injured *Salmonella*. Following pre-enrichment, the selective enrichment step destroys most of the competing flora, allowing the *Salmonella* to be recovered.

Muller[5] recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella*. Kauffmann[6] modified the formula to include oxbile and brilliant green as selective agents to suppress bacteria such as *Proteus* spp. Jeffries[7] described the addition of novobiocin at 40 mg per liter of broth to further suppress the growth of *Proteus* sp. Muller Kauffmann Tetrathionate Broth Base is used for isolating *Salmonella* from food, environmental samples[4,8-13] and animal feces.[14] Using more than one selective broth increases the isolation of *Salmonella* from samples with multiple serotypes.[15]

Principles of the Procedure

Muller Kauffmann Tetrathionate Broth Base contains peptone and beef extract as sources of carbon, nitrogen, vitamins and minerals. Oxgall and added brilliant green are selective agents which inhibit gram-positive and other gram-negative organisms. Calcium carbonate is the buffering agent. Sodium thiosulfate is a source of sulfur.

Formula

Difco™ Muller Kauffmann Tetrathionate Broth Base

Approximate Formula* Per Liter		
Beef Extract	5.0	g
Peptone	10.0	g
Sodium Chloride	3.0	g
Calcium Carbonate	45.0	g
Sodium Thiosulfate (anhydrous)	38.1	g
Oxgall	4.7	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Muller Kauffmann Tetrathionate Broth Base

Dehydrated Appearance:	Off-white to light beige, free-flowing, homogeneous.
Solution:	10.58% solution, insoluble in purified water. Solution is very pale green with white precipitate.
Prepared Appearance:	Very pale green with white precipitate.
Reaction of 10.58% Solution, with additives, at 25°C:	pH 7.0 ± 0.2 (adjusted)

Cultural Response

Difco™ Muller Kauffmann Tetrathionate Broth Base

Prepare the medium per label directions, with the addition of 1.9 mL iodine solution and 0.95 mL brilliant green solution per 100 mL of medium. Inoculate and incubate at 42-43°C for 18-24 hours. Subculture to Brilliant Green Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	10^3-2×10^3	None to poor	–
Proteus vulgaris	13315	10^3-2×10^3	None to poor	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	100-300	Good	Red
Salmonella senftenberg (NCTC 10384)		100-300	Good	Red

Directions for Preparation from Dehydrated Product

1. Suspend 105.8 g of the powder in 1 L of purified water and boil gently.
2. Cool to below 45°C.
3. Add 19 mL of iodine solution (20 g iodine and 25 g potassium iodide in 100 mL water) and 9.5 mL brilliant green solution (0.1 g brilliant green in 100 mL water).
4. Adjust the pH of the complete medium to 7.0 ± 0.2 using 1N HCl.
5. Dispense into sterile tubes, mixing well to evenly disperse the calcium carbonate.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Refer to appropriate references for details on sample collection and preparation according to sample type and geographic location.[4,8-13]

Consult appropriate references for details on test methods using Muller Kauffmann Tetrathionate Broth.[4,8-13]

Expected Results

Salmonella spp. will produce red colonies with good growth.

Limitations of the Procedure

1. The complete medium is unstable and should be used immediately. It may be stored at 2-8°C in the dark for no more than 7 days.
2. Due to the nutritional requirements and inhibitory characteristics of the organisms themselves, organisms other than salmonellae, such as *Morganella morganii* and some *Enterobacteriaceae* may grow in the medium.
3. Confirmatory tests, such as fermentation and seroagglutination reactions, should be carried out on all presumptive *Salmonella* colonies that are recovered.

References

1. Baird Parker. 1990. The Lancet. *336*:1231.
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4. Proietti, Pedrazzoli, Bosco, Galli, Canali and Franciosini. 2006. Presented at the Twelfth European Poultry Conference, Verona, Italy, 10 to 14 Sept. 2006.
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11. Electronic Code of Federal Regulations (e-CFR), Title 9: Animals and Animal Products, Part 147–Auxiliary provisions on national poultry improvement plan. 23 Dec 2008. <http://ecfr.gpoaccess.gov>.
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Availability

Difco™ Muller Kauffmann Tetrathionate Broth Base

Cat. No. 218531 Dehydrated – 500 g

Mycobacteria 7H11 Agar

(See Seven H11 Agars)

Mycobactosel™ L-J Medium

Intended Use

Mycobactosel L-J Medium is used in qualitative procedures for the isolation of mycobacteria from clinical specimens.

Summary and Explanation

Mycobactosel L-J Medium is Lowenstein-Jensen Medium plus cycloheximide, lincomycin and nalidixic acid for use with specimens likely to contain many contaminating organisms.[1-4] The authors, Petran and Vera, reported that the medium permits isolation of mycobacteria from a few more specimens and reduced by 68% the number of cultures which otherwise would have been reported as unsatisfactory as the result of overgrowth by contaminants.[5]

Principles of the Procedure

This medium contains a variety of inorganic salts or other nitrogen sources, which provide substances essential for the growth of mycobacteria. The glycerol is a source of carbon and energy. Asparagine is added to promote the initiation of growth and increase the growth rate. Egg yolk is a source of lipids for the metabolism of mycobacteria. Partial inhibition of bacteria is achieved by the presence of the malachite green dye.

Cycloheximide suppresses the growth of saprophytic fungi. Lincomycin inhibits gram-positive bacteria. Nalidixic acid inhibits some of the gram-negative bacteria encountered in clinical specimens.

Precaution[6]

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[7] N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle, but effective, digesting and decontaminating agent. These reagents are provided in the **BBL™ MycoPrep™** Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[7-10]

Media may be inoculated up to the expiration date and incubated for up to 8 weeks.

Following inoculation, keep test containers shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C.

Slanted media should be incubated in a horizontal plane until the inoculum is absorbed. Tubes should have screw caps loose for the first 3 weeks to permit circulation of carbon dioxide for the initiation of growth. Thereafter, to prevent dehydration, tighten caps; loosen briefly once a week. Stand tubes upright if space is a problem.

NOTE: Cultures from skin lesions suspected to be *M. marinum* or *M. ulcerans* should be incubated at 25-33°C for primary isolation; cultures suspected to contain *M. avium* or *M. xenopi* exhibit optimum growth at 40-42°C.[7] Incubate a duplicate culture at 35-37°C.[7]

Expected Results

Cultures should be read within 5 to 7 days after incubation and once a week thereafter for up to 8 weeks.

Record Observations[7]

1. Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7 days. Slow growers require more than 7 days for mature colony forms.
2. Pigment production
   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

1. Lowenstein. 1931. Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. I Orig. *120*:127.
2. Lowenstein. 1933. Ann. Inst. Pasteur. *50*:161.
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Availability

BBL™ Mycobactosel™ L-J Medium

CMPH2 MCM9

Cat. No.	221413	Prepared Slants (A Tubes) – Pkg. of 10*
	221414	Prepared Slants (A Tubes) – Ctn. of 100*

**Store at 2-8°C.*

Mycological Media
Mycological Agar • Mycophil™ Agar
Mycophil™ Agar with Low pH

Intended Use

Mycological media are used for the cultivation and maintenance of fungi, for the demonstration of chromogenesis and for obtaining yeast and mold counts.

Summary and Explanation

Many different culture media have been developed for the growth of fungi. In comparison with media for the majority of bacterial strains, fungal media are of simple composition, usually consisting of a peptone, dextrose and agar. Selectivity is achieved by lowering the pH, incorporating dyes or adding antimicrobial agents.

Mycological Agar and **Mycophil** Agar are nonselective media of value in general work with yeasts and molds rather than for isolation from materials possessing mixed flora. It is often desirable to use these media in parallel with selective media as some of the selective agents are inhibitory for certain fungi.

Mycophil Agar with Low pH has had its base adjusted to approximately pH 4.7, which obviates the need for pH adjustment with lactic or tartaric acids in the laboratory. It also differs from **Mycophil** Agar in that an additional 2 g/L of agar has been incorporated so that the medium may be sterilized and remelted without losing its ability to solidify.

Wetzler et al. employed **Mycophil** Agar with Low pH for enumeration of yeasts and molds in poultry processing plants.[1] The formulation also has been recommended for isolation of yeasts and most filamentous fungi from clinical material.[2]

Principles of the Procedure

The peptone and dextrose ingredients supply sufficient nutrients for the metabolism of fungal species.

Formulae

Difco™ Mycological Agar

Approximate Formula* Per Liter		
Soy Peptone	10.0	g
Dextrose	10.0	g
Agar	15.0	g

BBL™ Mycophil™ Agar

Approximate Formula* Per Liter		
Papaic Digest of Soybean Meal	10.0	g
Dextrose	10.0	g
Agar	16.0	g

BBL™ Mycophil™ Agar with Low pH

Approximate Formula* Per Liter		
Papaic Digest of Soybean Meal	10.0	g
Dextrose	10.0	g
Agar	18.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Mycological Agar

1. Suspend 35 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Mycophil™ Agar

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 118°C for 15 minutes.
4. For yeast and mold counts, adjust the pH to 4.0 by adding 15 mL of sterile 10% lactic acid to each L of sterile melted medium prior to plating.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Mycological Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Mycological Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C (incubate *Penicillium* at 20-25°C) for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	10231	30-300	Good
Penicillium abeanum	22346	30-300	Good
Saccharomyces cerevisiae	9080	30-300	Good
Staphylococcus aureus	25923	10^3-10^4	Good

Identity Specifications

BBL™ Mycophil™ Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain a large number of minute to small yellow specks.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.6% Solution at 25°C:	pH 7.0 ± 0.2

BBL™ Mycophil™ Agar with Low pH

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain tan specks.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.8% Solution at 25°C:	pH 4.7 ± 0.2

Cultural Response

BBL™ Mycophil™ Agar or Mycophil™ Agar with Low pH

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 25 ± 2°C for 7 days.

ORGANISM	ATCC™	RECOVERY MYCOPHIL™ AGAR	RECOVERY MYCOPHIL™ AGAR WITH LOW PH
Aspergillus brasiliensis (niger)	16404	Good	Good
Candida albicans	60193	Good	Good
Nocardia asteroides	19247	Good	N/A
Penicillium roquefortii	9295	Good	N/A
Penicillium roquefortii	10110	N/A	Good
Saccharomyces cerevisiae	9763	N/A	Good
Trichophyton mentagrophytes	9533	Good	N/A

BBL™ Mycophil™ Agar with Low pH

1. Suspend 38 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for about 30 seconds to completely dissolve the powder.
3. Autoclave at 118°C for 15 minutes or at 121°C for 10 minutes.
4. The medium should be cooled and used at once. If the medium is allowed to solidify after autoclaving, it may be remelted once. DO NOT OVERHEAT.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate plated media with test specimens or materials so as to obtain isolated colonies. Consult appropriate references for information about the processing and inoculation of specimens.[3,4] For isolation of fungi from potentially contaminated specimens, also inoculate a selective medium. Incubate plates at 25-30°C in an inverted position (agar side up) with increased humidity. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Expected Results

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Examine plates for fungal colonies exhibiting typical color and morphology. Yeast and mold colonies can be counted to determine the level of contamination in the test sample. Biochemical tests and serological procedures should be performed to confirm findings.[5]

References

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3. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
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Availability

Difco™ Mycological Agar
Cat. No. 240520 Dehydrated – 500 g

BBL™ Mycophil™ Agar
Cat. No. 211445 Dehydrated – 500 g

BBL™ Mycophil™ Agar with Low pH
Cat. No. 211450 Dehydrated – 500 g

Mycoplasma Media

(See PPLO Media)

Mycosel™ Agar

Intended Use

Mycosel Agar is a highly selective medium containing cycloheximide and chloramphenicol. It is recommended for the isolation of pathogenic fungi from materials having a large amount of flora of other fungi and bacteria.[1,2] BBL™ prepared plates of **Mycosel** Agar are deep-filled to reduce the effects of drying during prolonged incubation.

Summary and Explanation

Mycosel Agar was developed by using the ingredients of **Mycophil**™ Agar as a nutritive base to which cycloheximide and chloramphenicol were added as selective agents. It is widely used for the isolation of fungi from a variety of sources, and is recommended for the recovery of dermatophytes.[3]

User Quality Control

Identity Specifications

BBL™ Mycosel™ Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to moderately hazy.
Reaction of 3.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

BBL™ Mycosel™ Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 25 ± 2°C for 7 days.

ORGANISM	ATCC™	RECOVERY
Aspergillus brasiliensis (niger)	16404	Partial to complete inhibition
Aureobasidium pullulans	9348	Partial to complete inhibition
Blastomyces dermatitidis	56218	Good
Candida albicans	10231	Good
Escherichia coli	25922	Partial to complete inhibition
Microsporum audouinii	9079	Good
Penicillium roquefortii	9295	Partial to complete inhibition
Phialophora verrucosa	10223	Good
Staphylococcus aureus	25923	Complete inhibition
Streptomyces rimosus	10970	Partial to complete inhibition
Trichophyton mentagrophytes	9533	Good

Candida albicans
ATCC™ 10231

Principles of the Procedure

The nutritive properties of **Mycosel** Agar are supplied by the peptone prepared from soybean meal. Dextrose is an energy source for the metabolism of fungi. Cycloheximide inhibits most saprophytic molds. Chloramphenicol is a broad-spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria.

Formula

BBL™ Mycosel™ Agar

Approximate Formula* Per Liter		
Papaic Digest of Soybean Meal	10.0	g
Dextrose	10.0	g
Agar	15.5	g
Cycloheximide	0.4	g
Chloramphenicol	0.05	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation just until the medium boils, to completely dissolve the powder.
3. Autoclave at 118°C for 15 minutes. Avoid overheating.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for information about the processing and inoculation of specimens.[1-3]

For isolation of fungi from potentially contaminated specimens, a nonselective medium should be inoculated along with the selective medium. Incubate the containers at 25-30°C with increased humidity.

For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Expected Results

After sufficient incubation, the plates and **Mycoflask**™ bottles should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Examine containers for fungal colonies exhibiting typical color and morphology.[4] Biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Some fungi may be inhibited by the antibiotics in this medium.[5]

References

1. Weitzman, Kane and Summerbell. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (eds.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
3. Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
4. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.
5. Larone. 1995. Medically important fungi: a guide to identification, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Mycosel™ Agar

BS12 CMPH2 MCM9

Cat. No. 211462 Dehydrated – 500 g

United States and Canada

Cat. No.		
	221847	Prepared Plates (Deep Fill) – Pkg. of 20*
	220966	Prepared Tubed Slants (A Tubes) – Pkg. of 10*
	220967	Prepared Tubed Slants (A Tubes) – Ctn. of 100*
	297456	Prepared Tubed Slants (C Tubes) – Ctn. of 100*
	221130	**Mycoflask**™ Bottles – Pkg. of 10*
	221131	**Mycoflask**™ Bottles – Ctn. of 100*
	296233	Prepared 1 oz Bottles – Pkg. of 10*
	295698	Prepared 1 oz Bottles – Ctn. of 100*
	297718	Prepared 1 oz. Transgrow-style Bottles – Ctn. of 100*

Europe

Cat. No. 254417 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

NIH Thioglycollate Broth

(See Thioglycollate Media)

NZCYM Broth • NZYM Broth • NZY Broth with Thymine

Intended Use

NZCYM Broth, NZYM Broth and NZY Broth with Thymine are used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

NZCYM Broth was developed by Blattner et al. as an enriched medium for cultivating recombinant strains of *E. coli* and propagating λ bacteriophage.[1] *E. coli* grows rapidly in rich media, such as the NZ media, which provide amino acids, vitamins and other metabolites the cell would otherwise have to synthesize.[2]

User Quality Control

Identity Specifications

Difco™ NZCYM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.2% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 2.2% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ NZYM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 2.1% Solution at 25°C:	pH 7.0 ± 0.2

BBL™ NZY Broth with Thymine

Dehydrated Appearance:	Fine, homogeneous and free of extraneous material (may contain minute to small dark particles).
Solution:	2.1% solution, soluble in purified water. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 2.1% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ NZCYM Broth or NZYM Broth or BBL™ NZY Broth with Thymine

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli (C600)	23724	10^2-3×10^2	Good
Escherichia coli (K802)*	33526	10^2-3×10^2	Good

**For testing NZY Broth with Thymine.*

The three variations of NZ media allow the user to select a formulation appropriate to the need.

Principles of the Procedure

Casein digest, yeast extract, casamino acids and thymine provide the necessary nutrients and cofactors required for excellent growth of recombinant strains of *E. coli*. Due to its higher degree of digestion, casamino acids is an excellent source of free amino acids. Sodium chloride is included in the medium to provide a suitable osmotic environment. Magnesium sulfate/chloride is a source of magnesium ions required in a variety of enzymatic reactions, including DNA replication. Thymine is a growth factor supplement included for those genetic strains of *E. coli* that are unable to synthesize the chemical.

Formulae

Difco™ NZCYM Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Casamino Acids	1.0	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Magnesium Sulfate (anhydrous)	0.98	g

Difco™ NZYM Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Magnesium Sulfate (anhydrous)	0.98	g

BBL™ NZY Broth with Thymine

Approximate Formula* Per Liter		
Enzymatic Digest of Casein	10.0	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Magnesium Chloride (anhydrous)	0.94	g
Thymine	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve the powder in 1 L of purified water:
   Difco™ NZCYM Broth – 22 g;
   Difco™ NZYM Broth – 21 g;
   BBL™ NZY Broth with Thymine – 21.1 g.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult an appropriate reference for recommended test procedures.[3]

Expected Results
Growth should be evident in the form of turbidity.

References

1. Blattner, Williams, Blechl, Denniston-Thompson, Faber, Furlong, Grunwald, Kiefer, Moore, Schumm, Sheldon and Smithies. 1977. Science *196*:161.
2. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl(ed.). 1994. Current protocols in molecular biology, vol. 1. Current Protocols, New York, N.Y.
3. Sambrook and Russell. 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Availability
Difco™ NZCYM Broth
Cat. No. 240410 Dehydrated – 500 g

Difco™ NZYM Broth
Cat. No. 241510 Dehydrated – 500 g

BBL™ NZY Broth with Thymine
Cat. No. 299313 Dehydrated – 500 g

Neomycin Blood Agar

Intended Use
Neomycin Blood Agar is used for the isolation of group A streptococci (*S. pyogenes*) and group B streptococci (*S. agalactiae*) from clinical specimens in which the presence of these organisms is suspected.[1,2]

Summary and Explanation
Lancefield group A streptococci (*S. pyogenes*) and group B streptococci (*S. agalactiae*) are medically important gram-positive beta-hemolytic bacteria. Blanchette and Lawrence added neomycin to sheep blood agar to enhance the detection of group A and group B streptococci by suppressing the growth of other hemolytic organisms.[1] Most staphylococci, *Enterobacteriaceae*, *Neisseria*, *Listeria* and some *Pseudomonas* species are inhibited.

Principles of the Procedure
The nutrients in Neomycin Blood Agar are provided by peptones which are sources of nitrogenous compounds, carbon, sulfur and trace ingredients. The defibrinated sheep blood supplies enrichment for growth of fastidious organisms. Sodium chloride maintains osmotic equilibrium. The incorporation of 30 µg/mL of neomycin in this medium provides suppression of normal flora for improved recovery of the group A and group B streptococci.

Procedure
As soon as possible after the specimen is received in the laboratory, inoculate the specimen onto a Neomycin Blood Agar plate by firmly rolling swab over a third of the agar surface. Streak the remainder of the plate with a sterilized inoculating loop to obtain isolated colonies. Without re-sterilizing the loop, stab the agar two or three times in the area of heaviest inoculation.

Also, inoculate a **Trypticase**™ Soy Agar with 5% Sheep Blood (TSA II) plate to assure the recovery of other clinically significant microorganisms that may be inhibited on the selective medium.

Incubate inoculated plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide. Examine plates after 18-24 hours and 48-72 hours, if necessary.

Expected Results
After 18-48 hours of incubation, group A streptococci (*S. pyogenes*) will appear as translucent or opaque, white to gray, small (1-2 mm) colonies surrounded by a zone of beta hemolysis. A decrease in size as compared to the nonselective control is typical.

Neisseria spp., gram-negative rods and most beta-hemolytic staphylococci are inhibited. While both group A and group B streptococci will grow on this medium, susceptibility to bacitracin, utilizing **Taxo**™ A (0.04 unit) discs, may be used to differentiate them.

References

1. Blanchette and Lawrence. 1967. Am. J. Clin. Pathol. *48*:411.
2. Facklam and Washington. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Availability
BBL™ Neomycin Blood Agar

Unites States and Canada
Cat. No. 221792 Prepared Plates – Ctn. of 100*

Europe
Cat. No. 254444 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Bacto™ Neopeptone

Intended Use
Bacto Neopeptone is used in preparing microbiological culture media.

Summary and Explanation
Neopeptone is recommended for use in media for detection of fungi.[1] Apodaca and McKerrow[2] used neopeptone for the cultivation of *Trichophyton rubrum* for study of its proteolytic activity. Neopeptone has been cited as a component of culture media used for cultivation of human pathogens, notably, *Bordetella pertussis* and group A streptococci.

Neopeptone has also been reported to provide nutrients for support of spirochetes and protozoa. Wyss et al.[3] used neopeptone as a component of a medium for cultivation of *Treponema maltophilum* sp. nov., a fastidious oral anaerobe.

User Quality Control

Identity Specifications

Bacto™ Neopeptone

Dehydrated Appearance: Tan, free-flowing, granules.

Solution: 1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light to light amber, clear to very slightly opalescent, may have a precipitate. 2.0% solution is light to medium amber, clear to very slightly opalescent, may have a precipitate. 10.0% solution is medium to dark amber, slightly opalescent to opalescent, may have a precipitate.

Reaction of 1.0% Solution at 25°C: pH 6.9-7.5

Cultural Response

Biochemical Reactions

Bacto™ Neopeptone

Prepare a sterile solution of **Bacto** Neopeptone as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922	~10^7	Negative
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	0.1 mL, undiluted	Positive

Growth Response

Bacto™ Neopeptone

Prepare a sterile solution with 2% **Bacto** Neopeptone, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	30-300	Good
Staphylococcus aureus	25923	30-300	Good

Ifediba and Vanderberg[4] reported that neopeptone, in addition to calf serum, was used as an inexpensive replacement for human serum in cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Cushion and Ebbets[5] utilized neopeptone in their investigations of various media for cultivating *Pneumocystis carinii* without feeder cells. Optimal replication of *P. carinii* separated from host fungi cells was observed in media with neopeptone and N-acetylglucosamine at low pH.

Media formulations containing **Bacto** Neopeptone are specified in standard methods for multiple applications.[1,6-9]

Principles of the Procedure

Bacto Neopeptone is an enzymatic digest of protein. Neopeptone contains a wide variety of peptide sizes in combination with vitamins, nucleotides and minerals.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** Neopeptone in the formula of the medium being prepared. Add product as required.

Procedure

See appropriate references for specific procedures using **Bacto** Neopeptone.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Apodaca and McKerrow. 1990. J. Med. Vet. Mycol. *28*:159.
3. Wyss, Choi, Schupbach, Guggenheim and Gobel. 1996. Int. J. Syst. Bacteriol. *46*:745.
4. Ifediba and Vanderberg. 1980. J. Parasitol. *66*:236.
5. Cushion and Ebbets. 1990. J. Clin. Microbiol. *28*:1385.
6. Horowitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
7. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
9. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Bacto™ Neopeptone

AOAC BAM COMPF SMWW USDA

Cat. No. 211681 Dehydrated – 500 g
211680 Dehydrated – 10 kg

Neutralizing Buffer

Intended Use
Neutralizing Buffer is recommended for detection of microorganisms found on dairy and food equipment disinfected with chlorine or quaternary ammonium compounds.

Summary and Explanation
Neutralizing Buffer has the ability to inactivate the bactericidal and bacteriostatic effect of chlorine as well as quaternary ammonium compounds. Neutralizing Buffer is recommended for use in the microbiological examination of surfaces in standard methods for the examination of dairy products and foods.[1,2] Neutralizing Buffer is also recommended for the digestion and decontamination of mycobacterial specimens.[3]

User Quality Control

Identity Specifications
Difco™ Neutralizing Buffer

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	0.52% solution; soluble in purified water. Solution is very light to light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 0.52% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response
Difco™ Neutralizing Buffer

Prepare the buffer per label directions. Verify the neutralizing effect by diluting a quaternary ammonium compound with Neutralizing Buffer from 1:2,500 to 1:100,000. Inoculate tubes with *Staphylococcus aureus* ATCC™ 6538P. Prepare pour plates by transferring 1 mL from each dilution to Tryptone Glucose Extract Agar (Cat. No. 223000) and incubate at 32 ± 1°C for 42-48 hours. Observe for inactivation of the bactericidal activity as indicated by the growth pattern.

Principles of the Procedure
Monopotassium phosphate provides the buffering capability. Sodium thiosulfate inactivates the effect of chlorine compounds. The aryl sulfonate complex neutralizes the effects of quaternary ammonium compounds.

Formula
Difco™ Neutralizing Buffer

Approximate Formula* Per Liter	
Monopotassium Phosphate	42.5 mg
Sodium Thiosulfate	0.16 g
Aryl Sulfonate Complex	5.0 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Dissolve 5.2 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
See appropriate standard methods for specific test methodologies.[1-3]

Expected Results
Refer to appropriate references for results.

References

1. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.

Availability
Difco™ Neutralizing Buffer

CCAM COMPF SMD

Cat. No. 236210 Dehydrated – 100 g

New York City (NYC) Medium, Modified

Intended Use
New York City (NYC) Medium, Modified is a semi-transparent selective medium used in qualitative procedures primarily for the isolation of pathogenic *Neisseria*.

Summary and Explanation
Fauer et al., described a new medium, designated NYC medium, for the isolation of pathogenic *Neisseria* consisting of a peptone-corn starch-agar-phosphate buffered base supplemented with horse plasma, horse hemoglobin, dextrose, yeast dialysate and antimicrobics.[1,2] The **BBL™** formulation is modified by replacing the yeast dialysate and dextrose with **BBL™ IsoVitaleX™** Enrichment, a chemically defined supplement developed specifically to aid the growth of gonococci and other microorganisms,[3] and the agar content is reduced from approximately 20 to 13 g/L for improved growth of *N. gonorrhoeae* as reported by Anstey et al.[4]

The antimicrobial mixture in NYC Medium is similar to that of Martin-Lewis Agar, except that the vancomycin concentration is reduced from 4 to 2 µg/mL and trimethoprim is reduced from 5.0 to 3.0 µg/mL.[1,2] Both formulations contain anisomycin at 20 mg/L.

Clinical field trials with NYC Medium indicate that the medium is superior to both Thayer-Martin[1] and Martin-Lewis[5,6] agars in recovery of *N. gonorrhoeae*.

Principles of the Procedure

The peptones, horse plasma, hemoglobin and **IsoVitaleX** Enrichment in NYC Medium, Modified provide nutrients for the growth of *N. gonorrhoeae* and *N. meningitidis*. The phosphate salts buffer the medium at a neutral pH.

This selective medium contains the antimicrobial agents, vancomycin, colistin, anisomycin and trimethoprim, to suppress the normal flora. Vancomycin is active primarily against gram-positive bacteria. Colistin inhibits gram-negative bacteria, including *Pseudomonas* species, but is not active against *Proteus* species. Anisomycin inhibits yeasts. Trimethoprim inhibits *Proteus*.

Procedure

Streak the specimen as soon as possible after it is received in the laboratory. If material is being cultured directly from a swab, proceed as follows:[7]

1. Roll swab directly on the medium in a large "Z" to provide adequate exposure of swab to the medium for transfer of organisms.
2. Cross-streak the "Z" pattern with a sterile wire loop, preferably in the clinic. If not done previously, cross-streaking should be done in the laboratory.
3. Place the culture as soon as possible in an aerobic environment enriched with carbon dioxide.
4. Incubate at 35 ± 2°C and examine after overnight incubation and again after approximately 48 hours.
5. Subculture for identification of *N. gonorrhoeae* should be made within 18-24 hours. If shipped after incubation, colonies should be subcultured before performing biochemical identification tests in order to ensure that adequate viability is achieved.

Expected Results

Typical colonial morphology is as follows:

N. gonorrhoeae may appear as small (0.5-1.0 mm) grayish white to colorless mucoid colonies.
N. meningitidis appears as large colorless to bluish-gray mucoid colonies.

Colonies may be selected for Gram-staining, subculturing or other diagnostic procedures.[8]

References

1. Fauer, Weisburd, Wilson and May. 1973. Health Lab. Sci. *10*:44.
2. Fauer, Weisburd and Wilson. 1973. Health Lab. Sci. *10*:55.
3. Martin, Billings Hackney and Thayer. 1967. Public Health Rep. *82*:361.
4. Anstey, Gun-Munro, Rennie, Thornley, Schaus, Flannigan, Hussain and Maharajah. 1984. J. Clin. Microbiol. *16*:754.
5. Lawton and Koch. 1982. J. Clin. Microbiol. *20*:905.
6. Granato, Schneible-Smith and Weiner, 1981. J. Clin. Microbiol. *13*:963.
7. Center for Disease Control. 1975. Criteria and techniques for the diagnosis of gonorrhea, USPHS, Atlanta, Ga.
8. Knapp and Koumans. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ New York City (NYC) Medium, Modified

United States and Canada
Cat. No. 297173 Prepared Plates – Pkg. of 20*

Japan
Cat. No. 252139 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Niacin Assay Medium

Intended Use

Niacin Assay Medium is used for determining niacin concentration by the microbiological assay technique.

Meets *United States Pharmacopeia (USP)* performance specifications.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

User Quality Control

Identity Specifications

Difco™ Niacin Assay Medium

Dehydrated Appearance:	Off-white, homogeneous, tendency to clump.
Solution:	3.75% (single strength) or 7.5% (double strength) solution, soluble in purified water upon boiling 2-3 minutes. Single strength solution is very light amber, clear, may have a slight precipitate.
Prepared Appearance:	(Single strength) very light amber, clear, may have a slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Niacin Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus plantarum* ATCC™ 8014 when prepared in single strength and supplemented with nicotinic acid. The medium should produce a standard curve when tested using a nicotinic acid reference standard at 0.0 to 0.25 µg per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Niacin Assay Medium is prepared according to the formula described by Snell and Wright,[1] modified by Krehl, Strong and Elvehjem[2] and Barton-Wright.[3] Niacin Assay Medium is used in the microbiological assay of nicotinic acid or nicotinamide (niacin) using *Lactobacillus plantarum* ATCC™ 8014 as the test organism. The medium is specified in assay procedures published in the *USP*[4] and *Official Methods of Analysis of AOAC International* (AOAC).[5]

Principles of the Procedure

Niacin Assay Medium is a dehydrated medium free from nicotinic acid and its analogs but containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC™ 8014. The addition of nicotinic acid or its analogs in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Niacin Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Acetate	20.0	g
L-Cystine	0.4	g
DL-Tryptophan	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Thiamine Hydrochloride	200.0	µg
Calcium Pantothenate	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Biotin	0.8	µg
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 7.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volumes to 10 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

Procedure

Follow assay procedures as outlined in the *USP*[4] and AOAC[5] publications.

Stock cultures of the test organism *L. plantarum* ATCC 8014 are prepared by stab inoculation of Lactobacilli Agar AOAC or Micro Assay Culture Agar. After 24-48 hours incubation at 35-37°C, the cultures are kept refrigerated. Transfers are made in triplicate at monthly intervals.

The inoculum for assay is prepared by subculturing a stock culture of *L. plantarum* ATCC 8014 into 10 mL of Lactobacilli Broth AOAC or Micro Inoculum Broth. After 18-24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions and the supernatant decanted. The cells are washed three times with 10 mL sterile 0.85% saline. After the third wash, the cells are resuspended in 10 mL sterile 0.85% saline and finally diluted 1:100 with 0.85% sterile saline. One drop of this latter suspension is used to inoculate each 10 mL assay tube.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using niacin at levels of 0.0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 µg niacin per assay tube (10 mL). Niacin Assay Medium may be used for both turbidimetric and titrimetric analyses. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C. Titrimetric determinations are best made following 72 hours incubation at 35-37°C.

The concentration of niacin required for the preparation of the standard curve may be prepared by dissolving 0.05 g of niacin in 1,000 mL purified water, giving a stock solution of 50 µg per mL. Dilute the stock solution by adding 1 mL to 999 mL purified water (50 ng/mL). Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL of the 50 ng/mL solution per tube. Other standard concentrations may be used provided the standard falls within the limits specified by AOAC.[5]

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average and use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

References

1. Snell and Wright. 1941. J. Biol. Chem. *13*:675.
2. Krehl, Strong and Elvehjem. 1943. Ind. & Eng. Chem., Ann. Ed. *15*:471.
3. Barton-Wright. 1944. J. Biochem. *38*:314.
4. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia, 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
5. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Niacin Assay Medium

AOAC USP

Cat. No. 232210 Dehydrated – 100 g*

**Store at 2-8°C.*

Nitrate Broth

Intended Use

Nitrate Broth is recommended as an aid in the identification of aerobic and facultative anaerobic gram-negative microorganisms by means of the nitrate reduction test.

Summary and Explanation

Microorganisms may be differentiated according to their metabolism of certain substrates. The ability to reduce nitrate to nitrite is characteristic of the family *Enterobacteriaceae*.[1] Nonfermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable of denitrification, which is a reduction of nitrate to nitrogen gas. The production of gas from nitrate is an important differential test for glucose-nonfermenting gram-negative bacilli. The end product of reduction depends upon the bacterial species.[2]

Nitrate Broth is a basal medium containing potassium nitrate. The microorganism under evaluation is inoculated into the medium and after incubation, nitrate reduction may be determined. An inverted Durham fermentation tube in the prepared tubed medium serves to trap nitrogen gas produced through denitrification. The medium is evaluated for nitrate reduction by the addition of two reagents, Nitrate A Reagent (0.8% sulfanilic acid in 5N acetic acid) and Nitrate B Reagent (0.6% N, N-dimethyl-alpha-naphthylamine in 5N acetic acid), which detect the presence of a catabolic end product, and by the addition of Nitrate C Reagent, zinc dust, which detects the absence of remaining nitrate in the medium.[2]

User Quality Control

Identity Specifications

Difco™ Nitrate Broth

Dehydrated Appearance:	Light to medium tan, free-flowing, homogeneous.
Solution:	0.9% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 0.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Nitrate Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. Test for nitrate reduction using Nitrate A Reagent, Nitrate B Reagent and Nitrate C Reagent following label directions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	NITRATE REDUCTION
Acinetobacter calcoaceticus	19606	10^2-10^3	Good	–
Enterobacter aerogenes	13048	10^2-10^3	Good	+
Escherichia coli	25922	10^2-10^3	Good	+
Pseudomonas aeruginosa	27853	10^2-10^3	Good	+

Uninoculated Tube | *Acinetobacter calcoaceticus* ATCC™ 19606 | *Escherichia coli* ATCC™ 25922

Principles of the Procedure

Reduction of nitrate is generally an anaerobic respiration in which an organism derives its oxygen from nitrate. Depending upon environmental conditions, the end products of this metabolic process are usually not further oxidized or assimilated into cellular metabolism, but are excreted into the surrounding medium. *Enterobacteriaceae* characteristically reduce nitrate to nitrite which reacts with sulfanilic acid and N, N-dimethyl-alpha-naphthylamine to produce a red color (Griess reaction). The formation of other end products (ammonia, nitrous oxide, hydroxylamine, etc.) is also possible; therefore, the addition of zinc dust is used to detect unreduced nitrate. The formation of nitrogen gas, an end product typical of certain organisms, is evidenced by displacement of the medium from the Durham tube by the gas produced.[2]

Formula

Difco™ Nitrate Broth

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	4.0	g
Proteose Peptone No. 3	1.0	g
Potassium Nitrate	1.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 9 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Prior to inoculation of Nitrate Broth, the organism to be tested must have been previously isolated on some other suitable solid medium. The use of a pure culture is essential to correct performance of the test.

Using a sterile inoculating loop remove several similar isolated colonies from the agar medium and inoculate into a tube of Nitrate Broth. Replace cap loosely and incubate at 35-37°C.

Examine the tubes after 18-24 and 42-48 hours for growth and presence of gas in the Durham tube. After 24-48 hours add reagents as described in "Expected Results."

Expected Results

If growth is apparent after 24-48 hours of incubation, examine for presence of gas in the Durham tube. If gas is present and the test organism is a nonfermenter, the test is positive for denitrification (nitrate was reduced to nitrogen gas). If the organism is a fermenter, gas may or may not be present. Add 10 drops of Nitrate A Reagent and 10 drops of Nitrate B Reagent to the tube. Development of a red color within 2 minutes denotes a positive test for nitrate. If there is no color development, add a small amount (approximately 20 mg on the tip of an applicator stick) of Nitrate C Reagent. If no color develops within 5-10 minutes, nitrate was reduced beyond nitrite and the test is positive. The development of a red color indicates the presence of unreduced nitrate and the test is negative.

Limitations of the Procedure

1. Nitrate reduction is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, biochemical and serological tests. Appropriate texts should be consulted for additional information.[3-5]
2. Allow at least 2 minutes for the color to develop before considering the nitrate test negative.
3. The nitrate test is very sensitive. An uninoculated nitrate control should be tested with reagents to determine whether the medium is nitrate-free and that the glassware and reagents have not been contaminated with nitrous oxide.[2]
4. The addition of too much zinc dust may result in a false-negative reaction or just a fleeting color reaction.[6]

References

1. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
2. MacFaddin. 2000. Biochemical tests for the identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
4. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. Porres and Porter. 1974. Am. J. Med. Technol. *40*:257.

Availability

Difco™ Nitrate Broth

AOAC BAM COMPF ISO SMD USDA

Cat. No. 226810 Dehydrated – 500 g

BBL™ Nitrate Broth with Durham Tube

Cat. No. 221830 Prepared Tubes (K Tubes) – Pkg. of 10

Difco™/BBL™ Nitrate A Reagent

Cat. No. 261197 Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate B Reagent

Cat. No. 261198 Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate C Reagent

Cat. No. 261207 Droppers, 1 g – Ctn. of 50

Nocardia Differentiation Media
Casein Agar • Tyrosine Agar • Xanthine Agar
Starch Agar

Intended Use
These media are used in qualitative procedures for the differentiation of *Nocardia* species.

Summary and Explanation
Nocardiosis is a disease of man, most frequently in patients who are severely immunocompromised, and animals caused by *Nocardia* species.[1] The disease may resemble tuberculosis when the organism is inhaled, or may produce granulomatous abscesses when the organism is introduced into tissue at the time of an injury.

Biochemical tests demonstrating the hydrolysis of casein, tyrosine, xanthine and/or starch, or growth in 0.4% gelatin may be used to differentiate *Nocardia* species and to differentiate *Nocardia* from *Streptomyces* species, which appear morphologically similar to *Nocardia* in clinical materials and in culture.[1,2]

Agar deeps are provided in a 20 mL fill so that the media may be liquified and poured into Petri dishes. This provides a convenient source of medium with a longer shelf life than pre-poured plated media.

Principles of the Procedure
Casein Agar consists of agar with skim milk as a source of casein. Tyrosine Agar and Xanthine Agar consist of nutrient agar supplemented with tyrosine or xanthine. *Nocardia* may be differentiated from *Streptomyces* species based on patterns of enzymatic hydrolysis of casein, tyrosine and xanthine. Clear zones under colony growth and in the surrounding medium indicate hydrolysis of the substrate present.[1,2]

Starch Agar consists of nutrient agar supplemented with potato starch. *Nocardia* may be presumptively differentiated from *Streptomyces* based on the inability of *Nocardia* to hydrolyze starch. The hydrolysis of starch may be detected following incubation by flooding the agar surface with Gram's or Lugol's iodine. The medium surrounding the growth of *Nocardia* becomes temporarily colored dark blue, while the area surrounding the growth of *Streptomyces* remains unstained, indicating complete or partial hydrolysis of the starch.

A gelatin test can also be included to assist in the presumptive differentiation of *Nocardia brasiliensis* from *N. asteroides*. *N. brasiliensis* grows well in the 0.4% gelatin medium, producing compact, round colonies, while *N. asteroides* fails to grow or grows poorly and produces thin, flaky growth.

Procedure
Prepare a Gram-stained smear of the isolate and examine to confirm that the morphology is appropriate for *Nocardia* species. Subculture the isolate to be identified onto a Sabouraud Dextrose Agar slant or **Mycophil**™ Agar slant. Incubate at 25-30°C until good growth is observed.

To prepare plated media, place agar deeps with loosened caps in a boiling water bath until the medium becomes liquified (clear). Pour the molten medium into a sterile Petri dish. Allow the medium to solidify and dry before use. The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the Casein, Tyrosine, Xanthine or Starch medium by streaking the isolate to be tested onto the agar surface with a sterile inoculating loop. To prepare the 0.4% gelatin medium, add 0.4 g of gelatin to 100 mL of purified water warmed to 50-55°C in a water bath, dispense into tubes (4 mL/tube) and autoclave at 121°C for 15 minutes. Inoculate the gelatin medium just below the surface of the medium.

Incubate the media at 35°C.

Casein, Tyrosine and Xanthine Agars may need to be incubated for up to 3 weeks to allow positive hydrolytic reactions to develop. Examine plates at regular intervals for colony growth and hydrolytic reactions.

Incubate Starch Agar for 48-72 hours. Test for hydrolysis of the starch by flooding the agar surface with Gram's or Lugol's iodine. Observe for a purple color in the medium surrounding colony growth (a negative reaction). The medium does not change color if a positive reaction occurs.

Growth in 0.4% gelatin medium should be visible within 10 days of incubation.

Expected Results
The following table shows typical hydrolytic reactions that may be used to differentiate *Nocardia* and distinguish them from *Streptomyces*.

ORGANISM	CASEIN	TYROSINE	XANTHINE	STARCH	GELATIN
Nocardia asteroides	–	–	–	–	–
Nocardia brasiliensis	+	+	–	–	+
Nocardia caviae	–	–	+	–	–
Streptomyces spp.	+	+	+	+	+

References
1. Brown, McNeil and Desmond. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Larone. 1995. Medically important fungi: a guide to identification, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Casein Agar

Cat. No. 296188 Prepared Pour Tubes, 20 mL – Pkg. of 10*

BBL™ Tyrosine Agar

Cat. No. 297222 Prepared Pour Tubes, 20 mL – Pkg. of 10*

BBL™ Xanthine Agar

Cat. No. 297224 Prepared Pour Tubes, 20 mL – Pkg. of 10*

BBL™ Starch Agar

Cat. No. 297223 Prepared Pour Tubes, 20 mL – Pkg. of 10*

Difco™ Gelatin

Cat. No. 214340 Dehydrated – 500 g
214320 Dehydrated – 10 kg

**Store at 2-8°C.*

Nocardia ID QUAD

Intended Use

The Nocardia ID QUAD plate is a four-sectored plate containing four different media used for differentiation and identification of *Nocardia* species and other aerobic actinomycetes isolated from clinical specimens.

Summary and Explanation

The most frequently encountered aerobic actinomycetes, members of the order *Actinomycetales*, include the genera *Nocardia*, *Streptomyces*, *Actinomadura*, *Nocardiopsis*, *Rhodococcus* and *Dermatophilus*. The testing algorithm that permits identification of most aerobic actinomycetes consists of direct microscopic techniques and a minimum number of biochemical reactions.[1]

Quadrant I contains Casein Agar (skim milk and agar) for determining the ability of isolates to hydrolyze casein.

Quadrant II contains Starch Agar (potato starch and nutrient agar) for testing the ability of isolates to utilize starch.

Quadrant III contains Tyrosine Agar (tyrosine and nutrient agar) for testing the ability of isolates to decompose tyrosine.

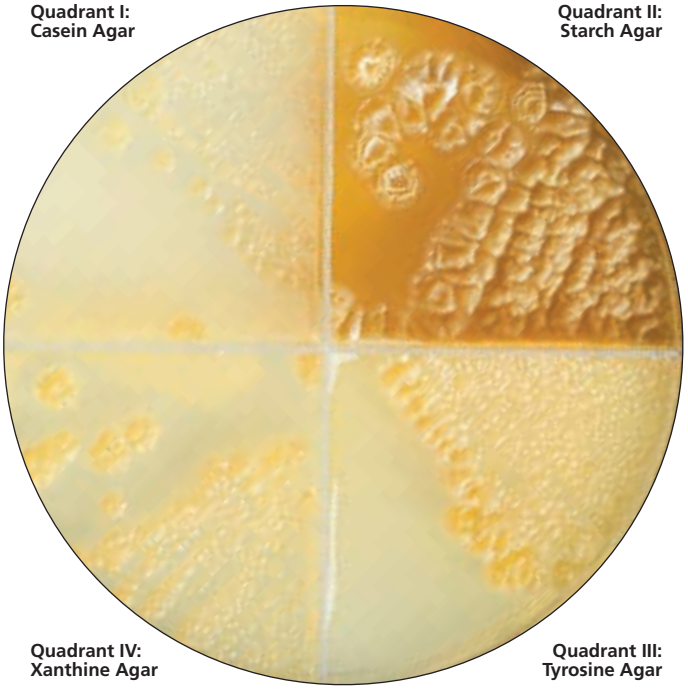
Quadrant IV contains Xanthine Agar (xanthine and nutrient agar) for testing the ability of isolates to decompose xanthine.

Principles of the Procedure

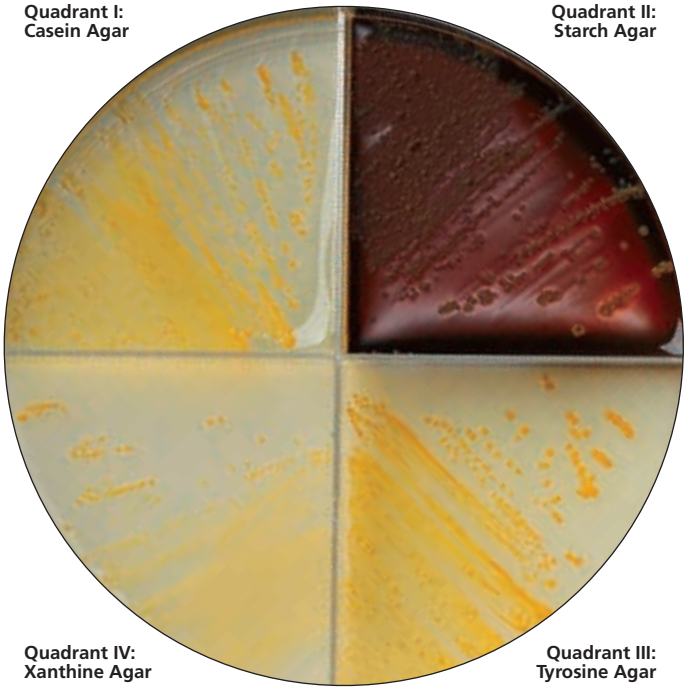
The four biochemical media contained in the Nocardia ID QUAD plates offer a convenient means of conducting four tests used in the differentiation and identification of *Nocardia* species following observation of staining reactions and microscopic characteristics.[1,2]

Decomposition of casein in Quadrant I may be detected by observing clear zones in the white, opaque skim milk around the inoculum. Growth without clearing around the inoculum is considered to be a negative test result.

The ability of isolates to break down and utilize starch may be detected in Quadrant II. Starch hydrolysis may be detected by colorless zones surrounding colonies after the plate is flooded



Streptomyces griseus
ATCC™ 10971



Nocardia asteroides
ATCC™ 19247

with Gram's iodine. Blue or purple zones surrounding colonies indicate a negative test.

The decomposition of tyrosine can be detected in Quadrant III. A clear halo around a colony is a positive test. Growth without the presence of clear halos or growth with the production of melanin-like pigment is a negative test.

The ability of isolates to decompose xanthine may be detected in Quadrant IV. A clear halo around a colony is a positive test. Growth without the presence of clear halos or growth with the production of a melanin-like pigment is a negative test.

Procedure

Inoculate each sector with a pure culture of the isolate. Use a small sterile spatula to obtain approximately 1 mm of the colony from a pure culture. Using the spatula, cut a small groove through the agar to the bottom of the plate, depositing the inoculum near the bottom of the groove. Alternatively, the tip of a sterile wooden applicator stick can be used to make a well through the agar to the bottom of the plate, depositing the inoculum at the bottom of the well.

Incubate the plates at 30°C in an inverted position (agar side up) under aerobic conditions and observe every 3-4 days for 14-21 days.[1]

Expected Results

Examine plates for growth periodically for 14-21 days of incubation.

Examine Quadrant I for the presence of a clear halo in the white opaque medium around the inoculum, which indicates a positive reaction. *N. brasiliensis* decomposes casein and gives a positive reaction. *N. asteroides* shows a negative reaction or no clearing around the inoculum.

To determine starch utilization, flood Quadrant II with Gram's or Lugol's iodine and observe the plate for colorless zones around the inoculum, which indicates a positive reaction, such as that obtained with *S. rimosus*. *N. asteroides* and *N. brasiliensis* give a negative reaction with no clear zones; blue or purple zones surround colonies.

The decomposition of tyrosine in Quadrant III is indicated by clear halos around colonies. There are no clear halos around colonies in a negative test. *N. brasiliensis* decomposes tyrosine, whereas *N. asteroides* does not.

The ability of isolates to decompose xanthine in Quadrant IV is shown by a clear halo around colonies, such as that obtained with *S. rimosus*. The absence of clear halos or the production of a melanin-like pigment indicates a negative test. Both *N. asteroides* and *N. brasiliensis* give a negative reaction.

References

1. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed., American Society for Microbiology, Washington, D.C.
2. Koneman, Allen, Janda, Schrechenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippencott-Raven Publishers, Philadelphia, Pa.

Availability

BBL™ Nocardia ID QUAD

BS12 CMPH2 MCM9

Cat. No. 298309 Prepared Plates (QUAD) – Pkg. of 10*

**Store at 2-8°C.*

Nutrient Agar

Intended Use

Nutrient Agar is used for the cultivation of bacteria and for the enumeration of organisms in water, sewage, feces and other materials.

Summary and Explanation

Early in the 20th century, the American Public Health Association published the formula for a general purpose medium for the growth of a wide variety of nonfastidious microorganisms.[1] This was in recognition of the need for a standardized medium for the use in the examination of water and wastewater, dairy products and various foods. This relatively simple formulation has stood the test of time, and with the name of Nutrient Agar, is still specified in current compendia of methods for the microbiological examination of a broad spectrum of materials.[2-5] Additionally, it is used in the laboratory for the cultivation and maintenance of nonfastidious species.

User Quality Control

Identity Specifications

Difco™ Nutrient Agar

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent.
Prepared Appearance:	Light amber, very slightly to slightly opalescent.
Reaction of 2.3% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Nutrient Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	19433	10^2-10^3	Good
Escherichia coli	25922	10^2-10^3	Good
Pseudomonas aeruginosa	27853	10^2-10^3	Good

Principles of the Procedure

Nutrient Agar consists of peptone, beef extract and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of microorganisms that are not excessively fastidious. The beef extract contains water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptones are the principle sources of organic nitrogen, particularly amino acids and long-chained peptides. Agar is the solidifying agent.

Formula

Difco™ Nutrient Agar

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Liquefy the agar if prepared tubes are used, cool to 45-50°C and pour into Petri dishes. Allow to solidify for at least 30 minutes. Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 35 ± 2°C for 18-24 hours and 42-48 hours, if necessary.

Tubed slants are used primarily for the cultivation and maintenance of pure cultures. They should be inoculated with an inoculating loop and incubated under the same conditions as the plated medium.

Expected Results

Examine plates for growth.

Growth from tubes inoculated with pure cultures may be used for biochemical and/or serological testing.

References

1. American Public Health Association. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, New York, N.Y.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
3. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
4. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Nutrient Agar

AOAC | BAM | CCAM | COMPF | ISO | SMWW | USDA

Cat. No.	212000	Dehydrated – 100 g
	213000	Dehydrated – 500 g
	211665	Dehydrated – 2 kg

BBL™ Nutrient Agar

AOAC | BAM | CCAM | COMPF | ISO | SMWW | USDA

United States and Canada

Cat. No.	297801	Prepared Plates – Pkg. of 10*
	220968	Prepared Pour Tubes – Pkg. of 10
	220971	Prepared Slants – Ctn. of 100

Mexico

Cat. No.	257500	Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Nutrient Agar 1.5%

Intended Use

Nutrient Agar 1.5% is used for cultivating a variety of microorganisms and with the addition of blood or other enrichment can be used for cultivating fastidious microorganisms.

Summary and Explanation

Nutrient Agar 1.5% is a modification of Nutrient Agar.[1] This medium is a slightly alkaline general-purpose medium. Since the medium contains 0.8% sodium chloride, it can be used as a base for enrichment with blood, ascitic fluid or other supplements for cultivating fastidious microorganisms.

Principles of the Procedure

Beef extract and peptone provide the nitrogen, vitamins, amino acids and carbon sources in Nutrient Agar 1.5%. Sodium chloride maintains the osmotic balance so that red blood cells will not rupture when blood is added as supplement.[1] Agar is the solidifying agent.

Formula

Difco™ Nutrient Agar 1.5%

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	5.0	g
Sodium Chloride	8.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.

User Quality Control

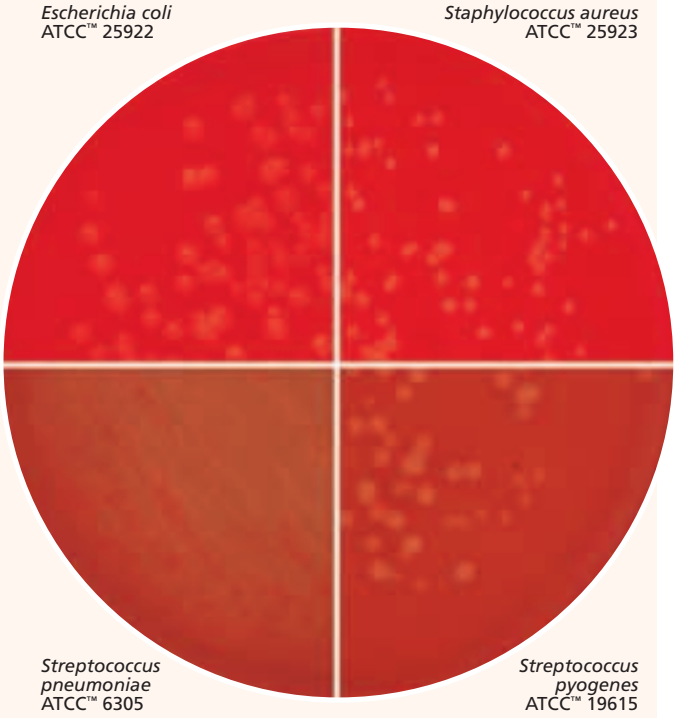
Identity Specifications
Difco™ Nutrient Agar 1.5%

Dehydrated Appearance:	Beige to light tan, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, very slightly to slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response
Difco™ Nutrient Agar 1.5%

Prepare the medium per label directions without (plain) and with 5% sheep blood (SB). Inoculate and incubate the plates at 35 ± 2°C for 40-48 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB	HEMOLYSIS
Escherichia coli	25922	10^2-10^3	Good	Good	Beta
Neisseria meningitidis	13090	10^2-10^3	Good	Good	Gamma
Staphylococcus aureus	25923	10^2-10^3	Good	Good	Beta
Streptococcus pneumoniae	6305	10^2-10^3	Good	Good	Alpha
Streptococcus pyogenes	19615	10^2-10^3	Good	Good	Beta



4. To prepare an enriched medium, cool the autoclaved base to 45-50°C and add the desired enrichment. Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
For a complete discussion of the isolation and identification of aerobic and anaerobic microorganisms, refer to appropriate references.

Expected Results
Refer to appropriate references and procedures for results.

Reference
1. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability
Difco™ Nutrient Agar 1.5%

COMPF

Cat. No. 269100 Dehydrated – 500 g

Nutrient Agar with MUG

Intended Use
Nutrient Agar with MUG is used for detecting and enumerating *Escherichia coli* in water.

Summary and Explanation
Escherichia coli is a member of the fecal coliform group of bacteria. The presence of *E. coli* is indicative of fecal contamination.[1] Feng and Hartman[2] developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) at a final concentration of 100 µg/mL into Lauryl Tryptose Broth. Nutrient Agar is similarly modified with the addition of MUG. Rapid quantitation and verification may be achieved with the membrane filtration procedure by transferring the membrane from a total-coliform or fecal-coliform positive sample to a Nutrient Agar substrate containing 4-methylumbelliferyl-β-D-glucuronide (MUG).[1]

Mates and Shaffer[3] used the membrane filter-Endo Agar method, followed by incubation on Nutrient Agar with MUG, to detect and enumerate *E. coli* within 4 hours of membrane transfer. *E. coli* was recovered at a rate of 98% with no false-positive results.

Nutrient Agar with MUG is prepared according to the formula specified by the U.S. Environmental Protection Agency[4] and published in *Standard Methods for the Examination of Water and Wastewater*.[1]

User Quality Control

Identity Specifications

Difco™ Nutrient Agar with MUG

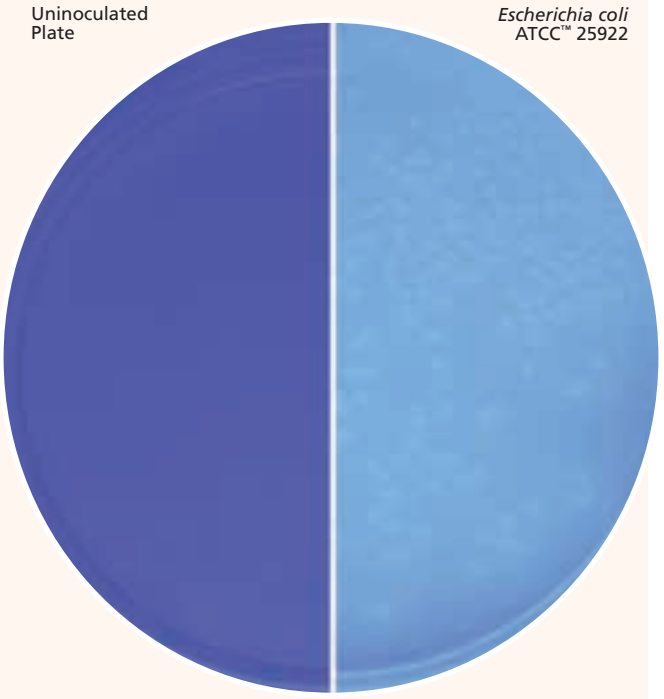
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.31% solution, soluble in purified water upon boiling. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to slightly opalescent.
Reaction of 2.31% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Nutrient Agar with MUG

Prepare the medium per label directions. After incubation on m Endo Agar LES using the membrane filter technique, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 4-24 hours at 35 ± 2°C. Examine for fluorescence under long-wave (approximately 366 nm) UV light.

ORGANISM	ATCC™	INOCULUM CFU	FLUORESCENCE
Enterobacter aerogenes	13048	30-300	–
Escherichia coli	25922	30-300	+



Principles of the Procedure

Beef extract and peptone are sources of nitrogen, vitamins, carbon and amino acids. Agar is the solidifying agent. The substrate, MUG (4-methylumbelliferyl-β-D-glucuronide), produces a blue fluorescence when hydrolyzed by the enzyme β-glucuronidase, which is produced by most *E. coli*.

Formula

Difco™ Nutrient Agar with MUG

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	5.0	g
Agar	15.0	g
MUG (4-Methylumbelliferyl-β-D-glucuronide)	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 23.1 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Follow the methods and procedures for water testing using m Endo Agar LES in standard methods.[1] After incubation on m Endo Agar LES, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 18-24 hours at 35 ± 2°C. Expose the filter surface to long-wave UV light.

Expected Results

Observe for fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence around the periphery of the colony under long-wave (approximately 366 nm) UV light.

Typical strains of *E. coli* (red with a green metallic sheen on m Endo Agar LES) exhibit blue fluorescence on Nutrient Agar with MUG. Non-*E. coli* coliforms may produce a metallic sheen but do not fluoresce.

Limitations of the Procedure

1. Glucuronidase-negative strains of *E. coli* have been encountered.[5-7] Similarly, MUG-negative strains of *E. coli* have been reported in this assay procedure but at a very low frequency.[3]
2. Strains of *Salmonella* and *Shigella* species that produce glucuronidase may infrequently be encountered.[8] These strains must be distinguished from *E. coli* on the basis of other parameters; i.e., gas production, lactose fermentation or growth at 44.5°C.

References

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Feng and Hartman. 1982. Appl. Environ. Microbiol. *43*:1320.
3. Mates and Shaffer. 1989. J. Appl. Bacteriol. *67*:343.
4. Federal Register. 1991. Fed. Regist. *56*:636.
5. Chang, Brill and Lum. 1989. Appl. Environ. Microbiol. *55*:335.
6. Hansen and Yourassowsky. 1984. J. Clin. Microbiol. *20*:1177.
7. Kilian and Bulow. 1976. Acta Pathol. Microbiol. Scand. Sect. B *84*:245.
8. Damare, Campbell and Johnston. 1985. J. Food Sc. *50*:1736.

Availability

Difco™ Nutrient Agar with MUG

EPA SMWW

Cat. No.	223100	Dehydrated – 100 g
	223200	Dehydrated – 500 g

Nutrient Broth

Intended Use
Nutrient Broth is used for the cultivation of many species of nonfastidious microorganisms.

Summary and Explanation
Nutrient Broth has the formula originally designed for use in the *Standard Methods for Examination of Water and Wastewater*. It is not a recommended bacteriological medium in later editions of this publication. It is one of several nonselective media useful in routine cultivation of microorganisms.[1-3]

Principles of the Procedure
This relatively simple formulation supports the growth of nonfastidious microorganisms due to its content of peptone and beef extract.

User Quality Control

Identity Specifications
Difco™ Nutrient Broth

Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 0.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response
Difco™ Nutrient Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good

Formula
Difco™ Nutrient Broth

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Dissolve 8 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Inoculate tubes of the broth medium with the test samples. Incubate tubes for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results
After incubation, growth is evidenced by the appearance of turbidity in the broth. Aliquots of the broth can be used for subculturing to solid media for purification and identification purposes.

References
1. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
3. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability
Difco™ Nutrient Broth

AOAC BAM CCAM COMPF SMD

Cat. No.	233000	Dehydrated – 100 g
	234000	Dehydrated – 500 g
	231000	Dehydrated – 2 kg
	232000	Dehydrated – 10 kg

BBL™ Nutrient Broth

BAM CCAM COMPF SMD

Cat. No.	221669	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10

Nutrient Gelatin

Intended Use
Nutrient Gelatin is used for the detection of gelatin liquefaction by microbial species.

Summary and Explanation
Nutrient Gelatin is made in accordance with the formula formerly used in the examination of water, sewage, and other materials of sanitary importance.[1] Gelatin liquefaction is one of the characteristics used in the classification of members of the *Enterobacteriaceae* and nonfermenting gram-negative bacteria. The use of Nutrient Gelatin for determining gelatin liquefaction patterns is considered to be the "standard" method for taxonomic studies, since the rate of liquefaction is important in the characterization of groups within the *Enterobacteriaceae* family as well as other groups of microorganisms.[2,3] Edwards and Ewing consider gelatin liquefaction to be an essential test for differentiation of enteric bacilli.[4]

Nutrient Gelatin is used chiefly for identification of pure cultures of bacteria that are not particularly fastidious in regard to nutritional requirements.

Principles of the Procedure

The peptone and beef extract supply sufficient nutrients for the growth of nonfastidious bacterial species. The gelatin is the substrate for the determination of the ability of an organism to produce gelatinases, which are proteolytic-like enzymes active in the liquefaction of gelatin.

Formula

Difco™ Nutrient Gelatin

Approximate Formula* Per Liter

Beef Extract	3.0	g
Peptone	5.0	g
Gelatin	120.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 128 g of the powder in 1 L of purified water.
2. Warm to 50°C to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Using a heavy inoculum (growth from an 18-24 hour pure culture), stab the tubes of Nutrient Gelatin with an inoculating needle directly down the center of the medium to a depth of approximately one-half an inch from the bottom of the tube.

User Quality Control

Identity Specifications

Difco™ Nutrient Gelatin

Dehydrated Appearance:	Tan, fine granular, free-flowing.
Solution:	12.8% solution, soluble in purified water upon warming in a 50-55°C water bath. Solution is light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 12.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Nutrient Gelatin

Prepare the medium per label directions. Stab inoculate using a heavy inoculum of fresh cultures and incubate at 35 ± 2°C for 1-7 days.

ORGANISM	ATCC™	RECOVERY	GELATINASE
Escherichia coli	25922	Good	–
Staphylococcus aureus	25923	Good	+

Incubate tubes, including an uninoculated control, at 35 ± 2°C for 24-48 hours and up to 14 days.

Expected Results

At various intervals during the incubation process, examine the tubes for growth (turbidity) and liquefaction. Use uninoculated control tubes for comparison. At each interval, tighten caps and transfer the tubes to a refrigerator or ice bath for a sufficient time period to determine whether liquefaction has or has not occurred. It is important that the tubes not be shaken during the transfer from incubator to refrigerator. When reading results, invert the chilled tubes to test for solidification or liquefaction.[3]

Consult appropriate texts for results with specific organisms.[3-6]

Limitations of the Procedure

1. This medium is not recommended for determination of gelatin liquefaction by fastidious species and obligate anaerobes.
2. Gelatin is liquid at temperatures above 20°C. If tubes are incubated at 35°C, they must be refrigerated in order to read for liquefaction. Include an uninoculated tube in the test procedure for comparison.
3. Growth and liquefaction frequently occur only at the surface of the tube. To prevent a false-negative interpretation, handle tubes carefully when warm so that liquified gelatin remains at the surface of the tube.[3]

References

1. American Public Health Association. 1960. Standard methods for the examination of water and sewage, 9th ed. American Public Health Association, New York, N.Y.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Nutrient Gelatin

USDA

Cat. No. 211100 Dehydrated – 500 g

BBL™ Nutrient Gelatin

USDA

Cat. No. 220974 Prepared Tubes, 8 mL (Deeps) – Pkg. of 10

OF Basal Medium • OF Medium with Carbohydrates

Intended Use

OF (Oxidation Fermentation) media are used for the determination of oxidative and fermentative metabolism of carbohydrates by gram-negative rods on the basis of acid reaction in either the open or closed system.

Summary and Explanation

OF Medium was developed by Hugh and Leifson who described the taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by gram-negative bacteria.[1] They showed that when an organism is inoculated into two tubes of OF Basal Medium containing a carbohydrate and the medium in one of the tubes is covered with melted petrolatum prior to incubation, the patterns of metabolism are of differential significance. Oxidative organisms only produce an acid reaction in the open tube with little or no growth and no acid formation in the covered tube. Fermentative organisms will produce an acid reaction in both types of tubes.

Changes in the covered agar are considered to be due to true fermentation, while changes in the open tubes are due to oxidative utilization of the carbohydrate present. If the carbohydrate is not utilized by either method, there is no acid production in either tube.

Principles of the Procedure

The medium contains a high concentration of added carbohydrates relative to the peptone concentration to avoid the utilization of peptone by an aerobic organism and the resultant production of an alkaline reaction which would neutralize slight acidity produced by an oxidative organism.[2] The dipotassium phosphate adds buffering capacity to the medium. The agar permits the determination of motility and aids in the even distribution of any acid produced at the surface of the medium.[3]

Dextrose is the most important carbohydrate for use in OF Basal Medium; however, certain organisms may metabolize other carbohydrates even if they are unable to utilize dextrose. Prepared tubed media containing arabinose, dextrose, dulcitol, fructose, galactose, lactose, maltose, mannose, raffinose, rhamnose, salicin, sorbitol, sucrose and xylose are provided.

Formula

Difco™ OF Basal Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	2.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	0.3	g
Bromthymol Blue	0.08	g
Agar	2.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ OF Basal Medium

Dehydrated Appearance:	Light beige with green tinge, free-flowing, homogeneous.
Solution:	0.94% solution, soluble in purified water upon boiling. Solution is green, clear to very slightly opalescent.
Prepared Appearance:	Green, clear to very slightly opalescent.
Reaction of 0.94% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ OF Basal Medium

Prepare the medium per label directions without (plain) and with 1% dextrose. Inoculate tubes in duplicate with fresh cultures using an inoculating needle and add an overlay of mineral oil to one set of tubes. Incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	PLAIN OPEN	PLAIN CLOSED	WITH DEXTROSE OPEN	WITH DEXTROSE CLOSED
Acinetobacter calcoaceticus	19606	K	K	A	K
Enterobacter aerogenes	13048	K	K	A, G	A, G
Escherichia coli	25922	K	K	A, G	A, G
Pseudomonas aeruginosa	27853	K	K	A	K
Shigella flexneri	12022	K	K	A	A

K = alkaline reaction, green medium
A = acid reaction, yellow medium
G = gas production

Uninoculated Tube | *Acinetobacter calcoaceticus* ATCC™ 19606 | *Escherichia coli* ATCC™ 25922 | *Pseudomonas aeruginosa* ATCC™ 27853

Tubes above are closed, with Dextrose

Directions for Preparation from Dehydrated Product

1. Suspend 9.4 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Add 1% carbohydrate before or after autoclaving depending on heat lability.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate a pair of OF tubes of each carbohydrate used with each organism being tested. The tubes should be stabbed to approximately 1/4 inch from the bottom using an inoculating needle and a light inoculum. Overlay one tube of each pair with sterile mineral oil. Incubate tubes at 35 ± 2°C in an aerobic atmosphere for 48 hours. Do not discard as negative until after 4 days of incubation.

Expected Results

Record results as acid (A) or alkaline/no change (–). Also record whether or not the organism is motile as evidenced by the appearance of growth away from the line of inoculation. Typical reaction patterns are as follows.[2-4]

Enteric OF Carbohydrate Utilization Patterns

REACTION	TUBE WITH REACTION	OPEN TUBE	COVERED TUBE
Oxidation (O)	Open	Yellow (A)	Green (–)
Fermentation (F)			
Anaerogenic	Covered	Yellow (A)	Yellow (A)
Aerogenic	Covered	Yellow (AG)	Yellow (AG)
Neither Oxidation nor Fermentation (–)	Neither*	Blue or Green (–)	Green (–)
Both Oxidation and Fermentation (O/F)	Both	Yellow (A or AG)	Yellow (A or AG)

A = acid production
G = gas production
– = no change or alkaline
** = Uninoculated carbohydrate control reading; no change in color.*

Limitations of the Procedure

1. The acid reaction produced by oxidative organisms is apparent at the surface and gradually spreads throughout the medium. If the oxidation is weak or slow, however, an initial alkaline reaction at the surface of the open tube may persist for several days and eventually convert to an acid reaction.
2. If an organism is unable to grow on OF Basal Medium, Cowan[5] recommends adding either 2% serum or 0.1% yeast extract to each carbohydrate tube.

References

1. Hugh and Leifson. 1953. J. Bacteriol. 66:24.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Shigei. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. Cowan. 1974. Cowan and Steele's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge, Mass.

Availability

Difco™ OF Basal Medium

BAM CCAM

Cat. No. 268820 Dehydrated – 500 g

BBL™ OF Basal Medium

Cat. No. 221326 Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*

BBL™ OF Basal Medium with Carbohydrates

Cat. No. 297783 Prepared Tubes with Arabinose – Pkg. of 10*
221328 Prepared Tubes with Dextrose – Pkg. of 10*
221329 Prepared Tubes with Dextrose – Ctn. of 100*
297784 Prepared Tubes with Dulcitol – Pkg. of 10*
297366 Prepared Tubes with Fructose (Levulose) – Pkg. of 10*
297785 Prepared Tubes with Galactose – Pkg. of 10*
221330 Prepared Tubes with Lactose – Pkg. of 10*
221332 Prepared Tubes with Maltose – Pkg. of 10*
221334 Prepared Tubes with Mannitol – Pkg. of 10*
297786 Prepared Tubes with Mannose – Pkg. of 10*
296374 Prepared Tubes with Raffinose – Pkg. of 10*
297368 Prepared Tubes with Rhamnose – Pkg. of 10*
297365 Prepared Tubes with Salicin – Pkg. of 10*
297367 Prepared Tubes with Sorbitol – Pkg. of 10*
221336 Prepared Tubes with Sucrose – Pkg. of 10*
221338 Prepared Tubes with Xylose – Pkg. of 10*

**Store at 2-8°C.*

OFPBL Agar

(See PC Agar)

OGYE Agar Base

Intended Use

OGYE Agar Base is for use with the antimicrobial agent, oxytetracycline, in isolating and enumerating yeasts and molds in foods.

Summary and Explanation

Acidified agar may be used for enumerating yeasts and molds in foods and dairy products. However, in some cases, antimicrobics better suppress bacterial growth and improve recovery of yeasts and molds.[1,2]

Mossel et al.[3,4] described Oxytetracycline-Glucose Yeast Extract (OGYE or OGY) Agar for selectively isolating and enumerating yeasts and molds in foods. Mossel et al. demonstrated improved recovery compared to acidified agar media.

OGYE Agar is specified as a standard methods medium for use with dairy products.[1]

Principles of the Procedure

OGYE Agar Base contains yeast extract to supply B-complex vitamins which stimulate growth. Dextrose is the carbon energy source. Agar is the solidifying agent. The addition of oxytetracycline inhibits the growth of bacteria.

Formula

Difco™ OGYE Agar Base

Approximate Formula* Per Liter

Yeast Extract	5.0	g
Dextrose	20.0	g
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 37 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add 100 mg of the antimicrobial agent, oxytetracycline, to the medium at 50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

User Quality Control

Identity Specifications

Difco™ OGYE Agar Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is medium amber, very slightly opalescent.
Prepared Appearance:	Medium amber, slightly opalescent.
Reaction of 3.7% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ OGYE Agar Base

Prepare the medium per label directions (with the addition of oxytetracycline). Inoculate using the pour plate technique and incubate at 22 ± 3°C for 48-72 hours (up to 5 days if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10^2-10^3	Good
Escherichia coli	25922	10^3-2×10^3	Inhibition
Saccharomyces cerevisiae	9763	10^2-10^3	Good
Saccharomyces cerevisiae	9080	10^2-10^3	Good

References

1. International Organization for Standardization. 2004. Milk and milk products – Enumeration of colony forming units of yeasts and/or moulds – colony count technique at 25°C. ISO 6611/IDF 94, 2004-10-15, 2nd ed. ISO, Geneva, Switzerland.
2. Beuchat and Cousin. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Mossel, Visser and Mengerink. 1962. Lab. Pract. *11*:109.
4. Mossel, Kleynen-Semmeling, Vincentie, Beerens and Catsaras. 1970. J. Appl. Bacteriol. *33*:454.

Availability

Difco™ OGYE Agar Base

CCAM COMPF IDF ISO

Cat. No. 218111 Dehydrated – 500 g

Oatmeal Agar

Intended Use

Oatmeal Agar is used for cultivating fungi, particularly for macrospore formation.

Summary and Explanation

Fungi are extremely successful organisms, as evidenced by their ubiquity in nature. Of the estimated 250,000 species, fewer than 150 are known primary pathogens of humans.[1]

Identification and classification of fungi is primarily based on the morphologic differences in their reproductive structures.[2] Fungi reproduce by producing spores.[2] Large, multi-celled spores are called macroconidia, macroaleuriospores or macrospores and are produced by aerial sporulation.[2]

The detection of fungi is a great concern in the pharmaceutical, food and cosmetic industry.

Principles of the Procedure

Oatmeal is a source of nitrogen, carbon, protein and nutrients. Agar is the solidifying agent.

Formula

Difco™ Oatmeal Agar

Approximate Formula* Per Liter

Oatmeal	60.0	g
Agar	12.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 72.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for specific procedures on the isolation and cultivation of fungi.

User Quality Control

Identity Specifications

Difco™ Oatmeal Agar

Dehydrated Appearance:	Beige, nonhomogeneous, may be slightly lumpy.
Solution:	7.25% solution, soluble in purified water upon boiling with frequent agitation. Solution is off-white, opaque with nonhomogeneous particles.
Prepared Appearance:	Off-white, opaque appearance with nonhomogeneous particles.
Reaction of 7.25% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Difco™ Oatmeal Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10^2-10^3	Good
Candida albicans	10231	10^2-10^3	Good
Saccharomyces cerevisiae	9763	10^2-10^3	Good

Expected Results

Refer to appropriate references and procedures for results.

References

1. Dixon, Rhodes and Fromtling. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.). Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.

Availability

Difco™ Oatmeal Agar

Cat. No. 255210 Dehydrated – 500 g

OP

Orange Serum Agar
Orange Serum Broth Concentrate 10×

Intended Use

Orange Serum Agar is used for cultivating aciduric microorganisms, particularly those associated with spoilage of citrus products.

Orange Serum Broth Concentrate 10× when diluted 1:10 is used for cultivating and enumerating microorganisms associated with spoilage of citrus products.

Summary and Explanation

The low pH of fruit juices makes citrus fruit products susceptible to spoilage by yeasts, molds and the bacteria *Lactobacillus* and *Leuconostoc*.[1] In the 1950s, Hays investigated spoilage in frozen concentrated orange juice. He found that an agar medium containing orange serum (juice) was superior to Lindegren Agar in isolating the microorganisms responsible for spoilage causing a buttermilk off-odor.[2] In a later comparative study, Murdock, Folinazzo and Troy found Orange Serum Agar, pH 5.4 to be a suitable medium for growing *Leuconostoc*, *Lactobacillus* and yeasts.[3] Stevens described preparation of dehydrated agar media containing orange serum.[4] The **BBL** formula for Orange Serum Agar differs only in a slightly increased orange serum content and in the incorporation of less agar.

Orange Serum Agar is included in recommended methods for examining fruit beverages.[1] Orange Serum Broth Concentrate 10× is used for small samples to initiate growth of saprophytic and pathogenic fungi,[5] as well as detecting and enumerating butyric acid anaerobes.[1]

Principles of the Procedure

Orange Serum Agar and Orange Serum Broth Concentrate 10× contain peptone as a source of carbon and nitrogen for general growth requirements. Orange serum provides the acid environment favorable to recovering acid-tolerant microorganisms. Yeast extract supplies B-complex vitamins which stimulate growth. Dextrose is the carbohydrate. Agar is the solidifying agent in Orange Serum Agar.

Formulae

BBL™ Orange Serum Agar

Approximate Formula* Per Liter

Orange Serum	10.0	g
Yeast Extract	3.0	g
Pancreatic Digest of Casein	10.0	g
Dextrose	4.0	g
Dipotassium Phosphate	2.5	g
Agar	15.5	g

Difco™ Orange Serum Broth Concentrate 10×

Approximate Formula* Per Ampule (100 mL)

Orange Serum	100.0	mL
Yeast Extract	30.0	g
Pancreatic Digest of Casein	100.0	g
Dextrose	40.0	g
Dipotassium Phosphate	25.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Orange Serum Broth Concentrate 10×

Concentrate Appearance:	Dark amber, clear solution.
Reaction of Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Difco™ Orange Serum Broth Concentrate 10×

Prepare the medium per label directions. Inoculate tubes and incubate for 18-48 hours 35 ± 2°C for *Lactobacillus fermentum* and 30 ± 2°C for the remaining organisms.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Aspergillus brasiliensis (niger)	16404	30-300	Growth
Lactobacillus fermentum	9338	30-300	Growth
Leuconostoc mesenteroides	23386	30-300	Growth
Saccharomyces cerevisiae	9763	30-300	Growth

Identity Specifications

BBL™ Orange Serum Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain dark tan particles.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan; clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan; clear to slightly hazy.
Reaction of 4.5% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

BBL™ Orange Serum Agar

Prepare the medium per label directions. Inoculate streak plates with fresh cultures and incubate for 66-72 hours at 30-32°C; for *Penicillium roquefortii,* incubate at 23-27°C. Inoculate pour plates with *Lactobacillus plantarum* and incubate for 66-72 hours at 30-32°C.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus gasseri	4962	Undiluted	Good
Leuconostoc mesenteroides	12291	Undiluted	Good
Penicillium roquefortii	10110	Undiluted	Good
Lactobacillus plantarum	8014	30-300	Good

Directions for Preparation from Dehydrated Product

BBL™ Orange Serum Agar

1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in quantities under 50 mL and autoclave at 121°C for 10 minutes. For larger quantities, increase the autoclave time. Avoid overheating with consequent darkening and poor solidification.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Orange Serum Broth Concentrate 10×

1. To prepare the single-strength medium, aseptically add 100 mL of Orange Serum Broth Concentrate 10× to 900 mL sterile purified water and mix thoroughly.
2. Aseptically dispense 10 mL amounts into sterile test tubes.

Procedure

BBL™ Orange Serum Agar

1. For the plate count method, prepare serial 10-fold dilutions of the test material.
2. Add 1 mL of test sample to a sterile Petri dish.
3. Add 18-20 mL of molten agar (cooled to 45-50°C) and swirl plate gently to mix well.
4. Allow to solidify before incubating at 30°C for 48 hours. Plates can be held up to 5 days.

Difco™ Orange Serum Broth Concentrate 10×

Orange Serum Broth Concentrate 10× diluted to single-strength is used for small samples to initiate growth.

Expected Results

BBL™ Orange Serum Agar

Record colony morphology for each type of growth.

Difco™ Orange Serum Broth Concentrate 10×

Turbidity indicates growth.

Limitations of the Procedure

1. Orange Serum Agar is not a differential medium. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.
2. If Orange Serum Agar is divided into aliquots and allowed to solidify, remelt only once. Repeated heating may produce a softer medium.

References

1. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. Hays. 1951. Proc. Fla. State Hortic. Soc. *54*:135.
3. Murdock, Folinazzo and Troy. 1952. Food Technol. 6:181.
4. Stevens. 1954. Food Technol. 8:88.
5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Orange Serum Agar

COMPF

Cat. No. 211486 Dehydrated – 500 g

Mexico

Cat. No. 252613 Prepared Plates (60 × 15 mm-style) – Pkg. of 20*

Difco™ Orange Serum Broth Concentrate 10×

COMPF

Cat. No. 251810 Ampule – 6 x 100 mL*

**Store at 2-8°C.*

Oxacillin Screen Agar

Intended Use

Oxacillin Screen Agar (originally named MRSA Screen Agar) was developed for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA). These strains are resistant to penicillinase-resistant penicillins (PRPs), such as methicillin, oxacillin and nafcillin. Since the method to detect MRSA uses the same inoculum as the Bauer-Kirby antimicrobial disc susceptibility test procedure, the oxacillin screen test may be conveniently performed on isolates at the same time as routine susceptibility testing.

Summary and Explanation

Resistance to penicillin in *S. aureus* was observed soon after the introduction of penicillin in the late 1940s.[1] By the late 1960s, methicillin/oxacillin-resistant strains of *S. aureus* began to be isolated in the United States.[2]

Three different resistance mechanisms contribute to oxacillin resistance in *S. aureus*. These are (1) the classic type, which involves production of a supplemental penicillin-binding protein (PBP) that is encoded by a chromosomal *mec*A gene, (2) hyper β-lactamase production, and (3) production of modified PBPs, which lowers the organism's affinity for β-lactam antibiotics.[3]

Staphylococcus aureus
ATCC™ 33592

Strains that possess the *mec* gene (classic resistance) are either homogeneous or heterogeneous in their expression of resistance. With homogeneous expression, virtually all cells express resistance when tested by standard *in vitro* tests. With heteroresistant expression, some cells appear susceptible and others appear resistant. Often, only 1 in 10^4 to 1 in 10^8 cells in the test population express resistance. Heterogeneous expression occasionally results in MICs that appear to be borderline; i.e., oxacillin MICs of 2 to 8 µg/mL. Isolates that have classic resistance are usually resistant to other agents such as erythromycin, clindamycin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, older fluoroquinolones, or aminoglycosides.[3]

Resistance mediated by hyper β-lactamase production or the presence of modified PBPs also results in borderline resistance. Isolates that are resistant by either hyper β-lactamase production or the modified PBP mechanism usually do not have multiple-drug resistance.[3] Additionally, **these isolates are unlikely to grow on the agar screen plate.**[4,5]

The methicillin-resistant population grows more slowly, prefers a lower temperature of incubation and a high salt concentration.

Principles of the Procedure

Mueller Hinton Agar is a medium that has been standardized for the disc diffusion procedure for antimicrobial susceptibility testing of aerobic bacteria.[6] Sodium chloride is added to improve the growth of the PRP-resistant sub-populations. Oxacillin is preferred for the detection of PRP resistance since it is more stable and results are more reliable.[7,8]

Procedure

1. Prepare the inoculum by suspending several well-isolated colonies of the *S. aureus* test isolate from an 18- to 24-hour plate culture into a tube of suitable broth medium, such as **Trypticase™** Soy Broth and adjust the turbidity to a 0.5 McFarland turbidity standard, or use the **BBL™ Prompt™** inoculation system.
2. Spot inoculate with 10 µL of test suspension using micropipette.
3. Alternatively, saturate a cotton swab with the test suspension and gently press out excess fluid against the inner wall of the tube. Streak plate by drawing swab over an approximately 1 inch (2.54 cm) area.

4. Include a **Trypticase** Soy Agar with 5% Sheep Blood (TSA II) plate as a nonselective growth control.
5. The test and control plates may be divided into several wedge-shaped sectors by marking the bottom of the plate. Several isolates may be tested on each plate. However, use and incubate each plate only once. DO NOT REUSE AND REINCUBATE a **BBL** Oxacillin Screen Agar plate.
6. Incubate plates at 30-35°C for a full 24 hours. **Do not exceed 35°C.**

Expected Results

Following incubation, observe plates for growth. Any growth, even one colony, indicates that the isolate is methicillin (oxacillin) resistant. No growth indicates that the organism is susceptible to PRPs (methicillin, nafcillin and oxacillin). Isolates that grow on Oxacillin Screen Agar should be reported as resistant to *all* β-lactam antimicrobial agents, including β-lactam/ β-lactamase inhibitor combinations and cephalosporins.

Limitations of the Procedure

1. Occasionally, *S. aureus* isolates with borderline resistant MICs may not grow within 24 hours. It is recommended that any equivocal results demonstrated on the screening plate be confirmed with a standard MIC test.
2. In-house studies have shown that there is a difference in inoculum size between inoculating with 10 µL of the test suspension using a micropipette and inoculating the plate with a swab. The likelihood of the emergence of the resistant sub-population is greater in a large population of bacterial cells. Detection of resistance, especially with the heterogeneous resistant population, is improved with the larger inoculum obtained by using a micropipette and inoculating the plate with 10 µL.[9]
3. Any isolate that grows on this medium should be tested quantitatively by broth or agar dilution to confirm oxacillin resistance and also resistance to other antimicrobial agents that are characteristic of MRSA, such as chloramphenicol, clindamycin, erythromycin, gentamicin and tetracycline.
4. The use of Oxacillin Screen Agar for the detection of methicillin/oxacillin resistant coagulase-negative staphylococci is not recommended.[7]

References

1. Chain, Florey and Jennings. 1949. *In* Florey, Chain, Heatley, Jennings, Sanders, Abraham and Florey (ed.), Antibiotics, vol. II. Oxford University Press, London.
2. Barrett, McGehee and Finland. 1968. N. Engl. J. Med. 279:444.
3. Swenson, Patel and Jorgensen. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Leitch and Boonlayangoor. 1994. *In* Isenberg (ed.), Clinical microbiology procedures manual, vol. 1 (suppl. 1). American Society for Microbiology, Washington, D.C.
5. Harberberger, Kallen, Driscoll and Wallace. 1998. Lab. Med. 29:302.
6. Clinical and Laboratory Standards Institute. 2006. Approved standard: M2-A9. Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.
7. Clinical and Laboratory Standards Institute. 2008. Disk diffusion supplemental tables, M100-S18 (M2). CLSI, Wayne, Pa.
8. Clinical and Laboratory Standards Institute. 2008. MIC testing supplemental tables: M100-S18 (M7). CLSI, Wayne, Pa.
9. Data on file, BD Diagnostics.

Availability

BBL™ Oxacillin Screen Agar

BS12 CLSI CMPH2 MCM9

United States and Canada
Cat. No. 221952 Prepared Plates – Pkg. of 10*

Europe
Cat. No. 254570 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Oxford Medium Base
Modified Oxford Antimicrobic Supplement

Intended Use

Oxford Medium Base is used to prepare Oxford Medium[1] or Modified Oxford Medium[2] for isolating and differentiating *Listeria monocytogenes*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[3] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[4] The first reported foodborne outbreak of listeriosis was in 1985.[5] Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[6]

Implicated vehicles of transmission include turkey frankfurters,[7] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[8]

Listeria spp. grow over a pH range of 4.4-9.6 and survive in food products with pH levels outside these parameters.[9] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[10]

User Quality Control

Identity Specifications

Difco™ Oxford Medium Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous (may contain small dark particles).
Solution:	5.75% solution, soluble in purified water upon boiling. Solution is medium amber, slightly to moderately opalescent with a blue ring at the surface of the liquid.
Prepared Appearance:	Light to medium amber, very slightly to slightly opalescent.
Reaction of 5.75% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Modified Oxford Antimicrobic Supplement

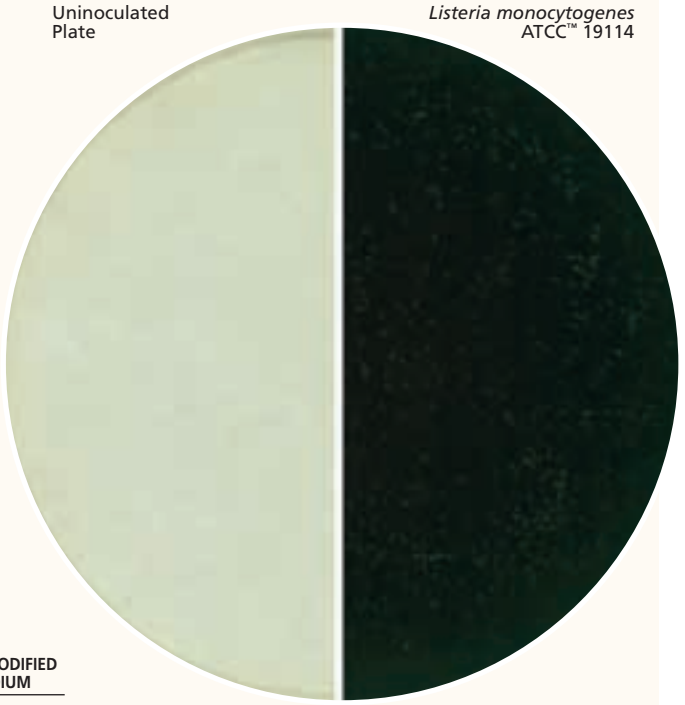
Appearance:	White cake may be broken; colorless solution with a pale yellow tint upon rehydration.

Cultural Response

Difco™ Oxford Medium or Modified Oxford Medium

Prepare the medium with corresponding supplement. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY ON OXFORD MEDIUM	RECOVERY ON MODIFIED OXFORD MEDIUM
Enterococcus faecalis	29212	10^3-2×10^3	Marked to complete inhibition	Marked to complete inhibition
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition	Marked to complete inhibition
Listeria monocytogenes	19114	10^2-10^3	Good at 40-48 hours, black colonies	Good at 40-48 hours, black colonies



OP

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Oxford Medium Base is prepared according to the formulation of Curtis et al.[11] who originally described the medium and its use in the selective isolation of *Listeria* from mixed cultures.

Principles of the Procedure

Peptones and beef heart digest provide nitrogen, carbon, amino acids and vitamins. Agar is the solidifying agent. Sodium chloride maintains the osmotic balance.

Ferric ammonium citrate aids in the differentiation of *Listeria* spp. Since all *Listeria* spp. hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the colony and surrounding medium in cultures containing esculin-hydrolyzing bacteria results from the formation of 6,7-dihydroxycoumarin which reacts with the ferric ions.[12]

Selectivity is provided by the presence of lithium chloride in the formula. The high salt tolerance of *Listeria* is used as a means to markedly inhibit growth of enterococci.

Selectivity is increased by adding various antimicrobial agents to the base. Incorporating these agents into Oxford Medium Base will completely inhibit gram-negative organisms and most gram-positive organisms after 24 hours of incubation. The most widely recognized antimicrobial agent combinations are the Oxford Medium formulation[11] and the Modified Oxford Medium formulation.[2] The Oxford Medium formulation contains cycloheximide, colistin sulfate, acriflavine, cefotetan and fosfomycin. The Modified Oxford Medium formulation contains moxalactam and colistin methane sulfonate or colistin sulfate (available as Modified Oxford Antimicrobic Supplement).

Modified Oxford Medium is recommended for isolating and identifying *Listeria monocytogenes* from processed meat and poultry products.[2] Oxford Medium is recommended for isolating *Listeria* from enrichment broth cultures.[13]

Formulae

Difco™ Oxford Medium Base

Approximate Formula* Per Liter

Pancreatic Digest of Casein	8.9	g
Proteose Peptone No. 3	4.4	g
Yeast Extract	4.4	g
Tryptic Digest of Beef Heart	2.7	g
Starch	0.9	g
Sodium Chloride	4.4	g
Esculin	1.0	g
Ferric Ammonium Citrate	0.5	g
Lithium Chloride	15.0	g
Agar	15.3	g

Difco™ Modified Oxford Antimicrobic Supplement

Formula Per 10 mL Vial

Colistin Sulfate	10.0 mg
Moxalactam	20.0 mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 57.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes. Cool to 45-50°C.
4. **To prepare Oxford Medium:** Dissolve acriflavine (5.0 mg), cefotetan (2.0 mg), colistin sulfate (20.0 mg), cycloheximide (400.0 mg) and fosfomycin (10.0 mg) in 5 mL reagant grade ethanol and 5 mL purified water. Filter sterilize supplement before use. Add 10 mL of antimicrobic supplement to 1 L of molten Oxford Medium Base (45-50°C). Mix thoroughly.
   To prepare Modified Oxford Medium: Aseptically rehydrate one vial of Modified Oxford Antimicrobic Supplement with 10 mL of sterile purified water. Rotate in an end-over-end motion to dissolve the contents completely. Add 10 mL of rehydrated Modified Oxford Antimicrobic Supplement to 1 L of Oxford Medium Base (45-50°C). Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The USDA method[2] involves enrichment of the food sample in UVM Modified Listeria Enrichment Broth (one part sample to nine parts broth) at 30°C. After incubation, a portion of the enrichment mixture is plated onto Oxford or Modified Oxford Medium.

The FDA method[1] involves adding 25 mL of liquid or 25 g of solid material to 225 mL Listeria Enrichment Broth and incubating at 30°C for 2 days. After enrichment, the broth is plated onto Oxford Medium.

For further information when testing food samples or clinical specimens for *Listeria*, consult appropriate references.[1,2,9,13,14]

Expected Results

Select esculin-positive colonies and confirm their identity by further biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification. For additional information, refer to appropriate references.[1,2,9,13,14]

Limitations of the Procedure

1. Since *Listeria* spp. other than *L. monocytogenes* can grow on these media, an identification of *L. monocytogenes* must be confirmed by biochemical and serological testing.[14]
2. Use freshly prepared antimicrobial agent solutions or aliquot portions and store at -20°C or below.
3. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

References

1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.
3. Murray, Webb and Swann. 1926. J. Pathol. Bacteriol. *29*:407.
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12. Fraser and Sperber. 1988. J. Food Prot. *51*:762.
13. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
14. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Oxford Medium Base

AOAC BAM CCAM COMPF ISO SMD USDA

Cat. No.	222530	Dehydrated – 500 g
	222510	Dehydrated – 2 kg

Difco™ Modified Oxford Antimicrobic Supplement

AOAC CCAM COMPF USDA

Cat. No.	211763	Vial – 6 × 10 mL*

**Store at 2-8°C.*

M-PA-C Agar

Intended Use

M-PA-C Agar is used for the selective recovery and enumeration of *Pseudomonas aeruginosa* from water.

Summary and Explanation

A variety of methods have been used for the enumeration of *P. aeruginosa* from water samples, some of which have been more widely accepted than others. The most-probable-number (MPN) procedures result in satisfactory recovery levels of *P. aeruginosa*, but are not usable for the testing of large-volume water samples and lack precision. These two deficiencies are eliminated in membrane filter (MF) techniques.

Many of the membrane filter media used for the recovery of *P. aeruginosa* lacked specificity and were of limited value when large heterogeneous microbial flora were present in the water samples. Levin and Cabelli devised *M*-PA Agar as a selective membrane filter medium for *P. aeruginosa*.[1] This formulation incorporated four antimicrobics, kanamycin, nalidixic acid, sulfapyridine and cycloheximide, which render the medium moderately selective. This original formulation was modified by raising the pH[2] and altering the content or concentration of ingredients.[3] The resulting medium was designated *M*-PA-B Agar.

Brodsky and Ciebin further modified these media by eliminating sulfapyridine and cycloheximide and produced *M*-PA-C Agar.[4] This formulation resulted in the ability to enumerate *P. aeruginosa* after only 24 hours of incubation at 41.5°C compared to 72 hours required with *M*-PA-B Agar and 96 hours for a presumptive MPN test.[4] *M*-PA-C Agar is identified as Modified *M*-PA Agar in *Standard Methods for the Examination of Water and Wastewater*.[5]

Principles of the Procedure

Yeast extract, lysine and the carbohydrates provide carbonaceous and nitrogenous compounds, energy sources and vitamins required for bacterial metabolism. Sodium chloride maintains osmotic equilibrium. The salts provide essential ions. Phenol red is a pH indicator, which becomes yellow in response to acids produced as a result of the fermentation of the carbohydrates. Kanamycin inhibits protein synthesis in gram-positive organisms.[6] Nalidixic acid blocks replication of susceptible gram-negative bacteria.[6]

Formula

BBL™ *M*-PA-C Agar

Approximate Formula* Per Liter		
Yeast Extract	2.0	g
L-Lysine HCl	5.0	g
Sodium Chloride	5.0	g
Xylose	1.25	g
Sucrose	1.25	g
Lactose	1.25	g
Phenol Red	0.08	g
Ferric Ammonium Citrate	0.8	g
Sodum Thiosulfate	5.0	g
Magnesium Sulfate	1.5	g
Kanamycin	8.0	mg
Nalidixic Acid	37.0	mg
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 35 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and pour into sterile 50-mm Petri dishes. Use the medium within 1 week after preparation.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Following filtration of the water sample through a sterile 47 mm, 0.45 µm gridded filter, place the membrane filter on the surface of a plate of *M*-PA-C Agar taking care to avoid the entrapment of bubbles between the agar and filter surface. Incubate for 72 hours at 41.5 ± 0.5°C in an aerobic atmosphere. Consult the standard method for additional information regarding the *M*-PA-C membrane filter technique.[5]

User Quality Control

Identity Specifications

BBL™ *M*-PA-C Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is medium to dark, orange-red to rose red, clear to slightly hazy.
Prepared Appearance:	Medium to dark, orange-red to rose red, clear to slightly hazy.
Reaction of 3.5% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

BBL™ *M*-PA-C Agar

Prepare the medium per label directions. Inoculate using the membrane filter technique and incubate at 41.5 ± 0.5°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	100-300	Partial to complete inhibition
Pseudomonas aeruginosa	10145	20-80	Good
Pseudomonas aeruginosa	27853	20-80	Good

Expected Results

Colonies on membrane filters are counted using a stereoscopic microscope at 10-15× magnification. Optimal colony density is 20-80 colonies. All colonies on the filter are counted when the density is 2 or fewer per square. The average of 10 squares is determined when the count is 3-10 colonies per square and the average of 5 squares is determined when the count is 10-20 colonies per square. Multiply the average count per square by 100 and divide by the sample volume to give colonies per milliliter.[5]

References

1. Levin and Cabelli. 1972. Appl. Microbiol. *24*:864.
2. Carson, Peterson, Favero, Doto, Collins and Levin. 1975. Appl. Microbiol. *30*:935.
3. Dutka and Kwan. 1977. Appl. Environ. Microbiol. *33*:240.
4. Brodsky and Ciebin. 1978. Appl. Environ. Microbiol. *36*:26.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
6. Estevez. 1984. Lab. Med. *15*:258.

Availability

BBL™ *M*-PA-C Agar

SMWW

Cat. No. 298153 Dehydrated – 500 g

PALCAM Medium Base
PALCAM Antimicrobic Supplement

Intended Use

PALCAM Medium Base is used with PALCAM Antimicrobic Supplement in isolating and cultivating *Listeria*, particularly from foods and milk products.

Summary and Explanation

PALCAM Medium Base and PALCAM Antimicrobic Supplement are based on the PALCAM agar formulation of van Nerren et al.,[1] who developed this selective and differential medium for use in the isolation and enumeration of *Listeria* spp. from food samples. PALCAM medium is widely recommended for use in the detection of *L. monocytogenes* in foods,[2-7] milk and milk products,[8] and environmental samples.[4]

Principles of the Procedure

Good growth of *Listeria* spp. is obtained by including Columbia Agar Base in PALCAM Medium Base. Columbia Agar Base provides the nutrients and cofactors required for good to excellent growth of *Listeria*. Selectivity of the complete medium is achieved through the presence of lithium chloride, polymyxin B sulfate and acriflavine HCl, present in PALCAM Medium Base, and ceftazidime, provided by PALCAM Antimicrobic Supplement. These agents effectively suppress growth of most commonly occurring non-*Listeria* spp. of bacteria present in foods. The ceftazidime concentration is reduced from 20 mg/L to 8 mg/L for improved growth and recovery of *Listeria*.

Differentiation on PALCAM Medium is based on esculin hydrolysis and mannitol fermentation. All *Listeria* spp. hydrolyze esculin as evidenced by a blackening of the medium. This blackening by esculin-hydrolyzing bacteria results from the formation of 6,7-dihydroxycoumarin, which reacts with ferric ions that are present in the medium as ferric ammonium citrate. On occasion, organisms other than *Listeria*, such as staphylococci or enterococci, may grow on this medium. Mannitol and the pH indicator, phenol red, have been added to differentiate mannitol-fermenting strains of these species from *Listeria* based on mannitol fermentation. Mannitol fermentation is demonstrated by a color change in the colony and/or the surrounding medium from red or gray to yellow due to the production of acidic end products.

User Quality Control

Identity Specifications

Difco™ PALCAM Medium Base

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	6.8% solution, soluble in purified water upon boiling. Solution is dark red, slightly opalescent.
Prepared Appearance:	Medium red, very slightly to slightly opalescent with slight precipitate.
Reaction of 6.8% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ PALCAM Antimicrobic Supplement

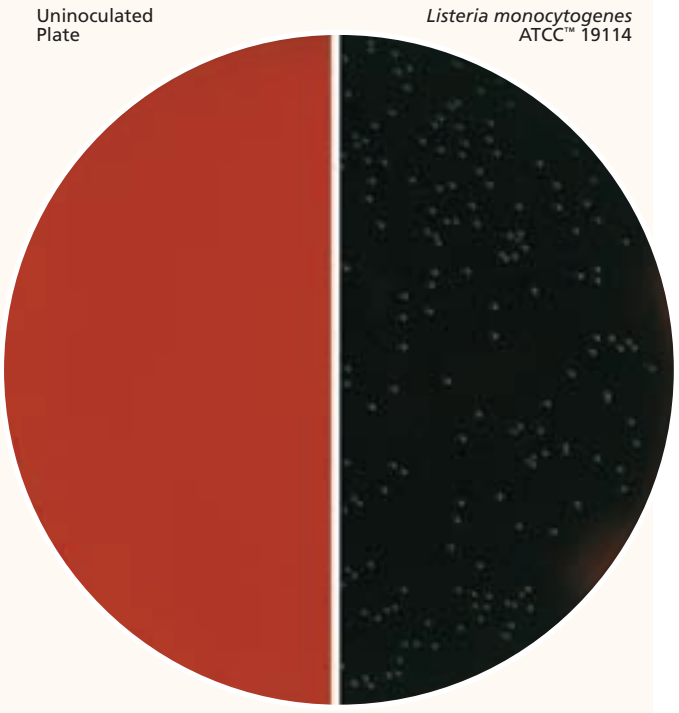
Lyophilized Appearance:	White, free-flowing, homogeneous powder.
Rehydrated Appearance:	Colorless solution.

Cultural Response

Difco™ PALCAM Medium Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours in a microaerophilic environment.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ESCULIN REACTION
Enterococcus faecalis	29212	10^3-2 × 10^3	Inhibition	–
Escherichia coli	25922	10^3-2 × 10^3	Inhibition	–
Listeria monocytogenes	19114	100-300	Good	+
Staphylococcus aureus	25923	10^3-2 × 10^3	Inhibition	–



Formulae

Difco™ PALCAM Medium Base

Approximate Formula* Per Liter		
Columbia Blood Agar Base	39.0	g
Pancreatic Digest of Casein	10.0	g
Proteose Peptone No. 3	5.0	g
Yeast Extract	5.0	g
Beef Heart, Infusion from 500 g	3.0	g
Corn Starch	1.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Mannitol	10.0	g
Dextrose	0.5	g
Esculin	1.0	g
Ferric Ammonium Citrate	0.5	g
Lithium Chloride	15.0	g
Phenol Red	0.08	g
Acriflavine HCl	5.0	mg
Polymyxin B Sulfate	0.01	g
Agar	2.0	g

Difco™ PALCAM Antimicrobic Supplement

Formula Per 10 mL Vial		
Ceftazidime	40.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ PALCAM Medium Base

1. Suspend 68 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 2 mL rehydrated PALCAM Antimicrobic Supplement. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.
6. Store the prepared medium at 2-8°C.

Difco™ PALCAM Antimicrobic Supplement

1. Aseptically add 10 mL sterile purified water to the vial.
2. Shake to dissolve the contents.
3. Upon rehydration, Difco PALCAM Antimicrobic Supplement is stable for 1 month when stored at 2-8°C.

Sample Collection and Handling

Follow appropriate standard methods for details on sample preparation and processing according to sample type and geographic location.[2-8]

Procedure

Consult appropriate references[2-8] and follow applicable standard methods. Inoculate incubated enrichment broth or screened food sample particle onto PALCAM Medium and streak for isolation. Incubate plates at 35°C for 24-48 hours under aerobic or microaerophilic conditions in an inverted position (agar side up).

Expected Results

On PALCAM Medium, colonies of *Listeria* appear gray-green with a black precipitate. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/serological identification.[2-8] Colonies of mannitol-fermenting organisms such as staphylococci, which may grow on this medium, appear yellow with a yellow halo.

References

1. Van Netten, Perales, Van de Moosalijk, Curtis, and Mossel. 1989. Int. J. Food Microbiol. *8*:299.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. Chapter 10: Detection and enumeration of *Listeria monocytogenes* in foods (January 2003). AOAC International, Gaithersburg, Md.
3. Downes and Ito (eds.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
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Availability

Difco™ PALCAM Medium Base

BAM CCAM COMPF ISO SMD

Cat. No.	263620	Dehydrated – 500 g
	263610	Dehydrated – 2 kg

Difco™ PALCAM Antimicrobic Supplement

BAM CCAM COMPF ISO SMD

Cat. No.	263710	Vial – 3 × 10 mL*

Europe

Cat. No.	254539	Prepared Plates (complete) – Pkg. of 20*

**Store at 2-8°C.*

PC Agar • OFPBL Agar

Intended Use

PC Agar and OFPBL Agar are used in the selective isolation and detection of *Burkholderia* (formerly *Pseudomonas) cepacia* from clinical and nonclinical specimens.

Summary and Explanation

Burkholderia cepacia is an opportunistic pathogen generally associated with nosocomial infections.[1] Studies indicate that *B. cepacia* may be an important pulmonary pathogen for patients with cystic fibrosis (CF).[1,2] The incidence of this organism in the respiratory tract of CF patients is often accompanied by rapid deterioration in pulmonary status and death.[3]

Recovery of this organism on commonly used media, such as blood agar or MacConkey Agar, is difficult because common

isolates, such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*, overgrow the slower-growing colonies of *B. cepacia* and mask its presence.

Gillian et al. developed PC Agar for improved recovery of *B. cepacia*.[2] Crystal violet, bile salts and two antimicrobial agents are used as selective agents. Phenol red facilitates detection of *B. cepacia* by a color change in the medium. They reported isolating *B. cepacia* on PC Agar from respiratory secretions of 35 CF patients, but isolated the organism from only 21 patients on MacConkey Agar.[2]

Welch et al. developed a differential but less selective medium for the recovery of *B. cepacia*.[4,5] This medium, OFPBL Agar, is OF (oxidation-fermentation) basal medium supplemented with polymyxin B, bacitracin, lactose and agar. The indicator, bromthymol blue, aids in the detection of *B. cepacia* isolates through a color change in the medium. These investigators reported isolating *B. cepacia* on OFPBL Agar from 58 CF patients, while only isolating this organism from 19 patients on MacConkey Agar.[4]

Principles of the Procedure

These media provide a variety of enzymatic digests of proteinaceous substrates, inorganic salts and other nutrients to satisfy the nutritional requirements of these organisms.

Selective agents are incorporated to improve the recovery of *B. cepacia* by inhibiting common contaminants. PC agar incorporates crystal violet to inhibit gram-positive cocci, especially enterococci and staphylococci, bile salts to inhibit most gram-positive cocci other than enterococci, and ticarcillin and polymyxin B to inhibit gram-negative bacilli. OFPBL Agar incorporates polymyxin B to inhibit gram-negative flora, while bacitracin inhibits the gram-positive organisms and *Neisseria*.[4]

PC Agar contains the pH indicator phenol red to facilitate detection of *B. cepacia*. Alkaline end products from the metabolism of pyruvate raise the pH of the medium, causing the color of the indicator to change from light orange to pink or pink-red in the area of growth. In areas of heavy growth of *B. cepacia*, the pink color intensifies.

OFPBL Agar contains the pH indicator bromthymol blue to facilitate detection of *B. cepacia*. Acid end products from the metabolism of lactose lower the pH of the medium resulting in a yellow color change. *B. cepacia* colonies will also have a yellow color.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar-side up) at 30-35°C for a minimum of 4 days to allow sufficient time for colony development and for the color of the indicator change.[6,7]

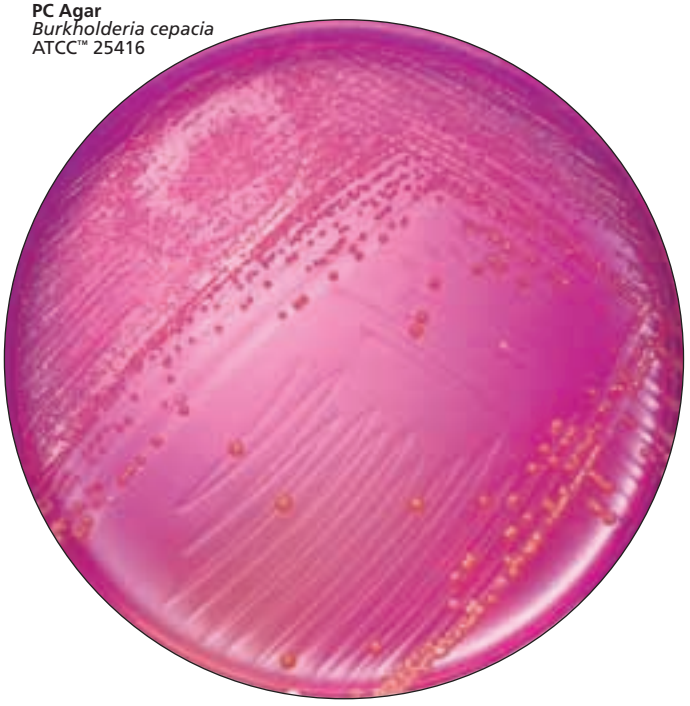
Expected Results

Typical colonies of *B. cepacia* on PC Agar are grayish-white with a pink-red zone in the surrounding medium.[2] Typical colonies of *B. cepacia* on OFPBL Agar are yellow with yellow zones in the surrounding medium.

Limitation of the Procedure

Organisms other than *B. cepacia* may also grow on PC Agar and produce alkaline end products that cause the medium to become pink. Other organisms, e.g., *B. gladioli*, may also grow on OFPBL Agar and resemble *B. cepacia* (yellow colonies). Therefore, these media should not be used as the sole method of identification of *B. cepacia*.[8]

PC Agar
Burkholderia cepacia
ATCC™ 25416



OFPBL Agar
Burkholderia cepacia
ATCC™ 25416

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Availability

BBL™ PC Agar

BS12 CMPH2 MCM9

Cat. No. 297755 Prepared Plates – Pkg. of 20*

BBL™ OFPBL Agar

BS12 MCM9

United States and Canada

Cat. No. 299970 Prepared Plates – Pkg. of 20*

Europe

Cat. No. 254481 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

OP

PPLO Media (Mycoplasma Media)
PPLO Agar (Mycoplasma Agar Base)
PPLO Broth (Mycoplasma Broth Base)
Mycoplasma Broth Base (Frey) • Mycoplasma Supplement • Mycoplasma Enrichment w/o Penicillin

Intended Use

PPLO (Mycoplasma) agars and broths, when supplemented with nutritive enrichments, are used for isolating and cultivating *Mycoplasma*. Mycoplasma Broth Base (Frey) is used for the cultivation of avian mycoplasmas.

Summary and Explanation

Members of the class *Mollicutes*, *Mycoplasma* was first recognized from a case of pleuropneumonia in a cow.[1] The organism was designated "pleuropneumonia-like organism," or PPLO.[1] Although some species are normal human respiratory tract flora, *M. pneumoniae* is a major cause of respiratory disease (primary atypical pneumonia, sometimes called "walking pneumonia").[1] *M. hominis*, *M. genitalium* and *Ureaplasma urealyticum* are important colonizers (and possible pathogens) of the human genital tract.[1]

PPLO (Mycoplasma) Agar was described by Morton, Smith and Leberman.[2] It was used in a study of the growth requirements of *Mycoplasma*,[3] along with the identification and cultivation of this organism.[4-6]

PPLO (Mycoplasma) Broth (without crystal violet) is prepared according to the formula described by Morton and Lecci.[3] Crystal violet is omitted from this formula due to its inhibitory action on some *Mycoplasma*. It has been used for the cultivation of *Mycoplasma* for research studies.[7,8]

Mycoplasma Broth Base (Frey), a modification of other broth media, was developed specifically for the cultivation of avian strains of *Mycoplasma*.[9]

Mycoplasma Supplement and Mycoplasma Enrichment w/o Penicillin are sterile desiccated enrichments for use in PPLO media as described by Hayflick.[10] The supplements are prepared according to the formulations of Chanock, Hayflick and Barile[11] and Hayflick.[12]

Principles of the Procedure

Meat digests, peptones, beef extract and yeast extract provide the nitrogen, vitamins, amino acids and carbon in these media. Sodium chloride maintains the osmotic balance of these formulations. Agar, the solidifying agent, is used in PPLO (Mycoplasma) Agar at a concentration slightly reduced from usual to ensure formation of the largest possible colonies because the organisms grow into the agar with only slight surface growth.[13]

The base media are supplemented with Mycoplasma Supplement or Mycoplasma Enrichment w/o Penicillin because *Mycoplasma* spp. are fastidious in their growth requirements.[14]

Mycoplasma Supplement contains fresh yeast extract and horse serum. Yeast extract provides the preformed nucleic acid precursors that are required by *Mycoplasma* spp.[14] Horse serum supplies cholesterol, a growth stimulant.[14]

Mycoplasma Enrichment without Penicillin is a selective enrichment containing the inhibitor thallium acetate, to which a penicillin of choice (penicillin G or a broad-spectrum semisynthetic penicillin) can be added at the time of use to make it selective against gram-positive and gram-negative bacteria.

Formulae

Difco™ PPLO Agar

Approximate Formula* Per Liter		
Beef Heart, Infusion from 50 g	6.0	g
Peptone	10.0	g
Sodium Chloride	5.0	g
Agar	14.0	g

Difco™ PPLO Broth

Consists of the the same ingredients without the agar.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ PPLO Agar (Mycoplasma Agar)

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is medium amber, slightly opalescent.
Prepared Appearance:	Enriched with 30% Mycoplasma Supplement–Light to medium amber, slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.8 ± 0.2

Difco™ PPLO Broth (Mycoplasma Broth)

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 2.1% Solution at 25°C:	pH 7.8 ± 0.2

Difco™ Mycoplasma Supplement

Desiccated Appearance:	Straw-colored, dried button, may be dispersed.
Rehydrated Appearance:	Light to dark straw-colored, clear to slightly opalescent.

Cultural Response

Difco™ PPLO Agar or PPLO Broth

Prepare the medium per label directions. Inoculate agar plates with 0.1 mL of serial dilutions of the test organisms. Incubate plates under 5-10% CO_2 at 35 ± 2°C for up to 7 days. Inoculate tubes of broth with 1.0 mL of serial dilutions of the test organisms and incubate under 5-10% CO_2 at 35 ± 2°C for up to 7 days, then subculture (0.1 mL) to plates of the agar medium and incubate under 5-10% CO_2 at 35 ± 2°C for up to 7 days. Daily examine plates microscopically for growth.

ORGANISM	ATCC™	RECOVERY
Mycoplasma arginini	23243	Good
Mycoplasma bovis	25523	Good
Mycoplasma gallinarum	19708	Good

BBL™ Mycoplasma Agar Base (PPLO Agar Base)

Approximate Formula* Per Liter

Beef Heart, Infusion from (solids)	2.0	g
Pancreatic Digest of Casein	7.0	g
Beef Extract	3.0	g
Yeast Extract	3.0	g
Sodium Chloride	5.0	g
Agar	14.0	g

BBL™ Mycoplasma Broth Base (PPLO Broth Base)

Consists of the same ingredients without the agar.

BBL™ Mycoplasma Broth Base (Frey)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	7.5	g
Papaic Digest of Soybean Meal	2.5	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Potassium Chloride	0.4	g
Magnesium Sulfate	0.2	g
Disodium Phosphate	1.6	g
Monopotassium Phosphate	0.1	g

Identity Specifications

BBL™ Mycoplasma Agar Base (PPLO Agar Base)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.4% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, trace hazy to hazy.
Prepared Appearance:	Light to medium, yellow to tan, trace hazy to hazy.
Reaction of 3.4% Solution at 25°C:	pH 7.8 ± 0.2

BBL™ Mycoplasma Broth Base (PPLO Broth Base)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water upon warming. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 7.8 ± 0.2

BBL™ Mycoplasma Broth Base (Frey)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.25% solution, soluble in purified water. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 2.25% Solution at 25°C:	pH 7.7 ± 0.2

BBL™ Mycoplasma Enrichment without Penicillin

Rehydrated Appearance:	Dark brown, clear to trace hazy.

Cultural Response

BBL™ Mycoplasma Agar Base or Mycoplasma Broth Base

Prepare the medium per label directions (enriched with **BBL** Mycoplasma Enrichment without Penicillin). Inoculate agar plates with 0.1 mL of serial dilutions of the test organisms. Incubate for 7 days at 35 ± 2°C with CO_2 for *Mycoplasma pneumoniae* and anaerobically for *Mycoplasma orale*. Inoculate tubes of broth with 1.0 mL of serial dilutions of the test organisms and incubate for 7 days at 35 ± 2°C with 3-5% CO_2 for *M. pneumoniae* and anaerobically for *M. orale*. After 5 days of incubation, subculture tubes (0.1 mL) to Mycoplasma Agar and incubate at 35 ± 2°C for up to 7 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	RECOVERY
Mycoplasma orale	23714	Good
Mycoplasma pneumoniae	1553	Good

BBL™ Mycoplasma Broth Base (Frey)

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 3-5% CO_2 for 7 days. Subculture to Mycoplasma Agar plates and incubate aerobically at 35 ± 2°C for 7 days. Examine plates microscopically for growth.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Growth in a dilution containing 10^3 CFU/mL
Mycoplasma gallisepticum	19610	Undiluted	Good
Mycoplasma synoviae	25204	Undiluted	Good

Difco™ Mycoplasma Supplement

Approximate Formula* Per 30 mL Vial		
Yeast Extract	0.09	g
Horse Serum	22.8	mL

BBL™ Mycoplasma Enrichment without Penicillin

Approximate Formula* Per 30 mL Vial		
Horse Serum	20.0	mL
Yeast Extract (fresh autolysate)	10.0	mL
Thallium Acetate	50.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ PPLO Agar
Difco™ PPLO Broth

1. **PPLO Agar:** Suspend 35 g of the powder in 700 mL of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
   PPLO Broth: Dissolve 21 g of the powder in 700 mL of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes. Cool medium to 50-60°C.
3. Aseptically add 300 mL **Difco** Mycoplasma Supplement to the medium. Mix well.
4. Add selective agents if desired (i.e., thallium acetate or penicillin).
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Mycoplasma Agar Base
BBL™ Mycoplasma Broth Base

1. **Mycoplasma Agar Base:** Suspend 34 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
   Mycoplasma Broth Base: Suspend 20 g of the powder in 1 L of purified water. Mix thoroughly. Warm slightly to completely dissolve the powder.
2. Autoclave at 121°C for 15 minutes.
3. Cool to 50°C and add enrichment. Recommended enrichments include addition of 20 mL of horse serum and 5 mL of specially prepared yeast extract[11] to each 75 mL of cooled medium.
4. For a selective medium inhibitory to bacteria, add 30 mL of **BBL** Mycoplasma Enrichment without Penicillin to 70 mL of molten agar medium (50°C) or 70 mL of broth medium and add sterile penicillin G to a final concentration of 500 units/mL.
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Mycoplasma Broth Base (Frey)

1. Dissolve 22.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes.
3. Cool to 50°C and add 100 mL of sterile inactivated horse serum. Mix thoroughly.
4. For recovery of *M. synoviae*, add 0.01% (w/v) nicotinamide adenine dinucleotide (NAD) and 0.01% (w/v) L-cysteine HCl. Inactivated swine serum is preferred in place of horse serum.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Mycoplasma Supplement
BBL™ Mycoplasma Enrichment without Penicillin

1. Rehydrate with 30 mL of sterile purified water.
2. Rotate gently to dissolve.
3. Add 30 mL (the contents of one vial) to 70 mL of sterile medium base.
4. Dispense in plates or tubes as desired.

Procedure

Agar

Inoculate the surface of plates containing the complete medium by adding drops of liquid inoculum or by a swab-inoculation technique. Incubate plates at 35 ± 2°C for up to 21 days in a moist atmosphere containing 5-10% carbon dioxide or anaerobically if the presence of *M. buccale*, *M. faucium*, *M. orale* or *M. salivarium* is suspected.[13]

Broth

Test material, either solid or liquid, should be directly inoculated into the broth medium. For preparation of stock organism suspensions, a block of agar culture can be added to the broth.

Following incubation at 35 ± 2°C in a moist aerobic atmosphere containing 5-10% carbon dioxide or anaerobically, if appropriate,[13] for various lengths of time, subculture aliquots of the broth to PPLO (Mycoplasma) Agar plates for visualization of typical colonies. The broth usually does not become turbid enough to confirm the presence of growth.

For a complete discussion of the isolation and identification of *Mycoplasma* spp. from clinical specimens, refer to appropriate procedures outlined in the references.[13-15]

Expected Results

Agar

PPLO colonies are round with a dense center and a less dense periphery, giving a "fried egg" appearance on PPLO (Mycoplasma) Agar. Vacuoles, large bodies characteristic of *Mycoplasma* spp., are seen in the periphery. Colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.

Broth

After subculture to plates of PPLO (Mycoplasma) Agar, positive broth cultures produce colonies exhibiting the typical morphology; i.e., "fried egg" appearance.

Limitation of the Procedure

Thallium acetate can partially inhibit some mycoplasmas.[13]

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Availability

Difco™ PPLO Agar (Mycoplasma Agar)

Cat. No. 241210 Dehydrated – 500 g

BBL™ Mycoplasma Agar Base (PPLO Agar Base)

Cat. No. 211456 Dehydrated – 500 g

Difco™ PPLO Broth (Mycoplasma Broth)

Cat. No. 255420 Dehydrated – 500 g
255410 Dehydrated – 10 kg

BBL™ Mycoplasma Broth Base (PPLO Broth Base)

Cat. No. 211458 Dehydrated – 500 g

BBL™ Mycoplasma Broth Base (Frey)

Cat. No. 212346 Dehydrated – 500 g
212347 Dehydrated – 5 lb (2.3 kg)

Difco™ Mycoplasma Supplement

Cat. No. 283610 Vial – 6 x 30 mL*

BBL™ Mycoplasma Enrichment w/o Penicillin

Cat. No. 212292 Vial – 10 x 30 mL*

**Store at 2-8°C.*

Pantothenate Assay Medium

Intended Use

Pantothenate Assay Medium is used for determining the concentration of pantothenic acid and its salts by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Pantothenate Assay Medium is a modification of the formula described in *The United States Pharmacopeia*[1] for the microbiological assay of calcium pantothenate using *Lactobacillus plantarum* ATCC™ 8014 as the test organism. Pantothenate Assay Medium does not contain polysorbate 80, which is included in **Difco**™ Pantothenate Medium AOAC.

Principles of the Procedure

Pantothenate Assay Medium is a dehydrated medium free from pantothenic acid or pantothenate but containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC 8014. The addition of calcium pantothenate in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Pantothenate Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	10.0	g
Dextrose	40.0	g
Sodium Acetate	20.0	g
L-Cystine	0.4	g
DL-Tryptophan	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Thiamine Hydrochloride	200.0	µg
Riboflavin	400.0	µg
Niacin	1.0	mg
Pyridoxine	800.0	µg
p-Aminobenzoic Acid	200.0	µg
Biotin	1.0	µg
Monopotassium Phosphate	1.0	g
Dipotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 7.3 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense in 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

Procedure

Prepare stock cultures of *L. plantarum* ATCC 8014 in triplicate by stab inoculation of Lactobacilli Agar AOAC. Incubate cultures for 18-24 hours at 35-37°C. Store the tubes at 2-8°C. Prepare a fresh stock culture every week. Do not use a culture older than 1 week for this assay.

Inoculum

Subculture from a stock culture of *Lactobacillus plantarum* ATCC 8014 to 10 mL of sterile single-strength Pantothenate Assay Medium supplemented with 0.02 µg pantothenate. Incubate for 18-24 hours at 35-37°C. Centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 mL sterile 0.85% saline. After the third wash, resuspend the cells with sterile 0.85% saline and adjust to a turbidity of 40-45% transmittance when read on a spectrophotometer at 660 nm. Aseptically inoculate each assay tube with one drop of the cell suspension.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated.

User Quality Control

Identity Specifications

Difco™ Pantothenate Assay Medium

Dehydrated Appearance:	Very light beige, homogeneous with a tendency to clump.
Solution:	3.65% (single-strength) solution, soluble in purified water upon boiling for 2-3 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	(Single strength) light amber, clear, may have a slight precipitate.
Reaction of 3.65% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Pantothenate Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus plantarum* ATCC™ 8014 when prepared in single strength and supplemented with pantothenic acid. The medium should produce a standard curve when tested with a pantothenic acid reference standard at 0.0 to 0.10 µg per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

The standard curve is obtained by using calcium pantothenate solution at levels of 0.0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08 and 0.1 µg per assay tube (10 mL). Turbidimetric determinations are made after 18-24 hours incubation at 35-37°C. Construct a standard curve and determine the concentration of the unknown by interpolation from the standard curve.

The concentration of pantothenic acid required for the preparation of the standard curve may be prepared by dissolving 50 mg dried calcium pantothenate in a solution containing approximately 500 mL purified water, 10 mL 0.2N acetic acid and 100 mL 0.2N sodium acetate. Dilute to 1,150 mL with additional water to make the calcium pantothenate concentration 43.47 µg per mL; one mL equals 40 µg pantothenic acid.

This solution is diluted by adding 25 mL to a solution containing 500 mL purified water, 10 mL 0.2N acetic acid and 100 mL 0.2N sodium acetate. Dilute to 1 liter with purified water to make a stock solution containing 1.0 µg pantothenic acid per mL. The standard solution is made by diluting 2 mL of the stock solution to 100 mL with purified water. This solution contains 0.02 µg pantothenic acid per mL. Use 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 mL per assay tube. Prepare the stock solution fresh daily.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

Reference

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Pantothenate Assay Medium

Cat. No. 260410 Dehydrated – 100 g*

**Store at 2-8°C.*

Pantothenate Medium AOAC

Intended Use

Pantothenate Medium AOAC is used for determining the concentration of pantothenic acid and pantothenate by the microbiological assay technique.

Meets *United States Pharmacopeia (USP)* performance specifications.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Pantothenate Medium AOAC is prepared for use in the microbiological assay of pantothenic acid and pantothenate according to the procedures of Calcium Pantothenate Assay in the *USP*[1] and Pantothenate Acid Assay in the *Official Methods of Analysis of AOAC International* (AOAC).[2] *Lactobacillus plantarum* ATCC 8014 is the test organism used in this assay.

User Quality Control

Identity Specifications

Difco™ Pantothenate Medium AOAC

Dehydrated Appearance:	White to very light beige, homogeneous, tendency to clump.
Solution:	3.65% (single strength) or 7.3% (double strength) solution,soluble in purified water upon boiling for 2-3 minutes. Single-strength solution is very light amber, clear, may have a slight precipitate.
Prepared Appearance:	(Single strength) light amber, clear, may have a very slight precipitate.
Reaction of 3.65% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Pantothenate Medium AOAC

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus plantarum* ATCC™ 8014 when prepared in single strength and supplemented with pantothenic acid. The medium should produce a standard curve when tested with a pantothenic acid reference standard at 0.0 to 0.05 µg per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Principles of the Procedure

Pantothenate Medium AOAC is a pantothenic acid/pantothenate-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *Lactobacillus plantarum* ATCC 8014. The addition of calcium pantothenate in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Pantothenate Medium AOAC

Approximate Formula* Per Liter		
Dextrose	40.0	g
Sodium Acetate	20.0	g
Vitamin Assay Casamino Acids	10.0	g
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
L-Cystine	0.4	g
L-Tryptophan	0.1	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Riboflavin	400.0	µg
Thiamine Hydrochloride	200.0	µg
Biotin	0.8	µg
p-Aminobenzoic Acid	200.0	µg
Nicotinic Acid	1.0	mg
Pyridoxine Hydrochloride	800.0	µg
Polysorbate 80	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 7.3 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the tube volume to 10 mL.
6. Autoclave at 121°C for 10 minutes.

Procedure

Follow the assay procedures as outlined in *USP*[1] or AOAC.[2]

Prepare stock cultures of *L. plantarum* ATCC 8014 by stab inoculation of Lactobacilli Agar AOAC. Incubate stock cultures at 35-37°C (± 0.5°C) for 18-24 hours. Store the stock cultures at 2-8°C. Prepare fresh stab cultures every week. Do not use a culture more than one week old for preparing the inoculum.

Subculture from a stock culture of *Lactobacillus plantarum* ATCC 8014 to a tube of sterile single-strength Pantothenate Medium AOAC (10 mL) supplemented with 0.2 µg pantothenate. Incubate for 18-24 hours at 35-37°C. Centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 mL sterile 0.85% NaCl. After the third wash, resuspend the cells with sterile 0.85% NaCl and adjust to a turbidity of 40-45% transmittance when read on a spectrophotometer at 660 nm. Aseptically inoculate each assay tube with one drop of the cell suspension.

Prepare solutions of Calcium Pantothenate USP Reference Standard or pantothenic acid (or equivalent) according to *USP*[1] or AOAC.[2] Satisfactory results are obtained with the standard curve by using pantothenic acid at levels of 0.0, 0.005, 0.01, 0.015, 0.02 and 0.025 µg per assay tube (10 mL) for the AOAC procedure. Calcium pantothenate may be used at standard levels of 0.0, 0.01, 0.02, 0.03, 0.04 and 0.05 µg per assay tube for the *USP* procedure. Pantothenate Medium AOAC may be used for both turbidimetric and titrimetric analysis in the AOAC procedure, and for turbidimetric analysis only for the *USP* procedure. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C (± 0.5°C). Titrimetric determinations are made following 72 hours incubation at 35-37°C (± 0.5°C).

The concentration of pantothenic acid or calcium pantothenate required for the preparation of the standard curve may be prepared as follows:

1. Dissolve 50 mg dried calcium pantothenate in 500 mL purified water, 10 mL 0.2N acetic acid and 100 mL 0.2N sodium acetate.
2. Dilute with additional water to make calcium pantothenate concentration 43.47 µg per mL for the AOAC procedure or dilute to 50 µg per mL for the *USP* procedure. At 43.47 µg per mL, one mL should equal 40 µg pantothenic acid.

Dilute further by adding 25 mL of this solution to 500 mL purified water, 10 mL 0.2N acetic acid and 100 mL 0.2N sodium acetate. Dilute this solution to 1 liter with purified water to make a stock solution containing 1 µg pantothenic acid per mL. The standard solution is made by diluting 5 mL of the stock solution to 1000 mL with purified water to obtain a solution containing 0.005 µg pantothenic acid per mL. Use 0.0, 1, 2, 3, 4 and 5 mL per assay tube. For the *USP* procedure, dilute the 50 µg per mL solution with purified water to make a standard concentration of 0.01 µg per mL. Other standard concentrations may be used provided the standard falls within the limits specified by *USP*[1] and AOAC.[2]

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ± 10% from the average and use the results only if two-thirds of the values do not vary more than ± 10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Horwitz (ed). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Pantothenate Medium AOAC

AOAC USP

Cat. No. 281610 Dehydrated – 100 g*

**Store at 2-8°C.*

Peptic Digest Agar

Intended Use
Peptic Digest Agar is used for the cultivation of *Haemophilus* species from a variety of clinical specimens.

Summary and Explanation
Peptic Digest Agar consists of Brain Heart Infusion Agar supplemented with Fildes Enrichment and **BBL™ IsoVitaleX™** Enrichment. Brain Heart Infusion Agar has proven to be effective in the cultivation of a wide variety of microorganisms including many types of pathogens. When supplemented with Fildes and **IsoVitaleX** Enrichments, it is used for the cultivation of fastidious organisms; e.g., *Haemophilus influenzae*.

Principles of the Procedure
Peptic Digest Agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusions are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is an energy source. The medium is buffered through the use of disodium phosphate.

Fildes Enrichment is a peptic digest of sheep blood that supplies the X (hemin) and V (nicotinamide adenine dinucleotide, NAD) factors necessary for the growth of *H. influenzae*.[1] **IsoVitaleX** Enrichment is a defined supplement which provides V factor (NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of fastidious microorganisms; e.g., pathogenic *Neisseria*.[2]

Procedure
Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 35°C in a CO_2-enriched aerobic atmosphere for 18-72 hours.

Expected Results
H. influenzae colonies are small, moist and grayish with a characteristic "mousy" odor. Gram staining, biochemical tests and other procedures should be performed to confirm findings.

References
1. Fildes. 1920. Br. J. Exp. Pathol. *1*:29.
2. Vastine, Dawson, Hoshiwara, Yonega, Daghfous and Messadi. 1974. Appl. Microbiol. *28*:688.

Availability
BBL™ Peptic Digest Agar

Cat. No. 297705 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Bacto™ Peptone

Intended Use
Bacto Peptone is used as an organic nitrogen source in microbiological culture media for cultivation of a variety of bacteria and fungi.

Summary and Explanation
Bacto Peptone was first introduced in 1914 and became the standard peptone for the preparation of bacteriological culture media. **Bacto** Peptone is used as an organic nitrogen source in microbiological culture media for cultivation of a variety of bacteria and fungi. For example, Iwanaga et al.[1] utilized **Bacto** Peptone for production of cholera toxin by *Vibrio cholerae* O1 El Tor. Benkerroum et al.[2] reported using **Bacto** Peptone in a selective medium developed for isolating *Leuconostoc* sp. from food samples. **Bacto** Peptone was used in a culture medium for two anaerobic, extremely thermophilic Archaea, *Thermococcus celer* and *Pyrococcus woesei*, by Blamey et al.[3]

Bacto Peptone has also been utilized as a nitrogen source in cell culture media formulations. Taylor et al.[4] used **Bacto** Peptone to supplement serum-free medium for several mammalian cell lines and reported that the solubility of **Bacto** Peptone is very good at 10 g/100 mL water. Sakoda and Fukusho[5] also utilized **Bacto** Peptone in serum-free culture medium for maintaining porcine kidney epithelial cells. **Bacto** Peptone is also useful as a supplement in cell culture with serum.

Researchers uncovered estrogenic activity associated with **Bacto** Peptone when including the peptone in medium for culture of yeast. The estrone contained in **Bacto** Peptone was converted to estrodiol by *Saccharomyces cerevisiae*. These findings suggest that adding estrogens to a medium containing **Bacto** Peptone for studies of estrodiol production by yeast may confound results.[6,7]

Several media containing peptone are specified in standard methods for multiple applications.[8-15]

Principles of the Procedure
Bacto Peptone is an enzymatic digest of animal protein. **Bacto** Peptone contains nitrogen in a form that is readily available for bacterial growth. **Bacto** Peptone has a high peptone and amino acid content, with only a negligible quantity of proteoses and more complex nitrogenous constituents.

Typical Analysis
Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product
Refer to the final concentration of **Bacto** Peptone in the formula of the medium being prepared. Add appropriate product as required.

User Quality Control

Identity Specifications

Bacto™ Peptone

Dehydrated Appearance:	Tan, free-flowing, granules.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is light amber, clear. 2.0% solution is light to medium amber, clear. 10.0% solution is medium to dark amber, clear to very slightly opalescent, may have a very slight precipitate.
Reaction of 1.0% Solution at 25°C:	pH 6.8-7.2

Cultural Response

Biochemical Reactions

Bacto™ Peptone

Prepare a sterile solution of **Bacto** Peptone as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922	~10^7	Negative
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	0.1 mL, undiluted	Positive

Growth Response

Bacto™ Peptone

Prepare a sterile solution with 2% **Bacto** Peptone, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	30-300	Good
Staphylococcus aureus	25923	30-300	Good

Procedure

See appropriate references for specific procedures using Bacto Peptone.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Iwanaga, Yamamoto, Higa, Ichinose, Nakasone and Tanabe. 1986. Microbiol. Immunol. *30*:1075.
2. Benkerroum, Misbah, Sandine and Elaraki. 1993. Appl. Environ. Microbiol. *59*:607.
3. Blamey, Chiong, Lopez and Smith. 1999. J. Microbiol. Methods. *38*:169.
4. Taylor, Dworkin, Pumper and Evans. 1972. Exp. Cell Res. *74*:275.
5. Sakoda and Fukusho. 1998. In Vitro Cell. Dev. Biol. Anim. *34*:53.
6. Feldman and Krishnan. 1995. Environ. Health Perspect. 103 Suppl 7:129.
7. Miller, Bottema, Stathis, Tokes and Feldman. 1986. Endocrinology. *119*:1362.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
9. Horowitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
10. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
11. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
12. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: *Enterococci* and *Escherichia coli*. EPA-821/R-97/004. Office of Water, Washington, D.C.
13. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
14. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
15. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Bacto™ Peptone

AOAC BAM COMPF EP EPA SMD SMWW USDA USP

Cat. No.	211677	Dehydrated – 500 g
	211820	Dehydrated – 2 kg
	211830	Dehydrated – 10 kg

Peptone Iron Agar

Intended Use

Peptone Iron Agar is used for detecting hydrogen sulfide production by microorganisms.

Summary and Explanation

Levine and co-workers[1,2] described a medium containing proteose peptone and ferric citrate for detection of hydrogen sulfide production by coliform bacteria. They demonstrated that such a medium served to differentiate strains that were Voges-Proskauer negative, methyl-red positive and citrate positive from other members of the *Enterobacteriaceae*.

User Quality Control

Identity Specifications

Difco™ Peptone Iron Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 3.6% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Peptone Iron Agar

Prepare the medium per label directions. Stab inoculate tubes with fresh cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	H_2S PRODUCTION
Enterobacter aerogenes	13048	Good	–
Escherichia coli	25922	Good	–
Proteus vulgaris	6380	Good	+
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	Good	+

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Salmonella* Enteritidis ATCC™ 13076

Levine reported that ferric citrate was a much more sensitive indicator of hydrogen sulfide production than lead acetate, producing a medium that gave definite reactions within 12 hours. Peptone Iron Agar is a modification of Levine's original formula in which peptone has been included with proteose peptone and the more soluble ferric ammonium citrate is used in place of ferric citrate.

Tittsler and Sandholzer[3] compared Peptone Iron Agar with lead acetate agar for the detection of hydrogen sulfide and found that Peptone Iron Agar had the advantage of giving earlier reactions and clearer results.

Principles of the Procedure

Peptones are the nitrogen sources in Peptone Iron Agar. Ferric ammonium citrate and sodium thiosulfate are used to detect H_2S production. Sodium glycerophosphate is a buffering compound. Agar is the solidifying agent.

Formula

Difco™ Peptone Iron Agar

Approximate Formula* Per Liter

Peptone	15.0	g
Proteose Peptone	5.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Glycerophosphate	1.0	g
Sodium Thiosulfate	0.08	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Obtain a pure culture of a test organism. Pick the center of a single colony with an inoculating needle.
2. Inoculate a tube of Peptone Iron Agar by the stab method. Stab the needle to within 1/4 to 1/2 inch of the bottom. Withdraw the needle following the initial line of inoculation.
3. Incubate tubes at 35 ± 2°C for 18-48 hours.
4. Read tubes for growth and hydrogen sulfide production.

Expected Results

Any blackening of the medium along the line of inoculation or throughout the butt indicates hydrogen sulfide production.

For a complete discussion of the identification of coliform bacteria, refer to appropriate references.[4-6]

References

1. Levine, Vaughn, Epstein and Anderson. 1932. Proc. Soc. Exp. Biol. Med. 29:1022.
2. Levine, Epstein and Vaughn. 1934. Am. J. Public Health 24:505.
3. Tittsler and Sandholzer. 1937. Am. J. Public Health 27:1240.
4. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Peptone Iron Agar

Cat. No. 289100 Dehydrated – 500 g

Peptone Water

Intended Use

Peptone Water is used for cultivating nonfastidious organisms, for studying carbohydrate fermentation patterns and for performing the indole test.

Summary and Explanation

The formulation of Peptone Water makes it useful for cultivating nonfastidious organisms.[1] This nonselective medium has been used as a basal medium for biochemical tests such as carbohydrate fermentation patterns and production of indole.[1,2]

Principles of the Procedure

Peptone Water contains peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium.

User Quality Control

Identity Specifications

Difco™ Peptone Water

Dehydrated Appearance:	Cream-white to light tan, free-flowing, homogeneous.
Solution:	1.5% solution, soluble in purified water upon warming with frequent agitation. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to slightly opalescent.
Reaction of 1.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Peptone Water

Growth/Indole Reaction

Prepare the medium per label directions. Inoculate with a fresh culture and incubate at 35 ± 2°C for 18-48 hours. Indole reaction is read using the **BBL™ DrySlide™** Indole test slide (Cat. No. 231748).

ORGANISM	ATCC™	RECOVERY	INDOLE REACTION
Escherichia coli	25922	Good	Positive

Carbohydrate Fermentation

Prepare the medium per label directions with the addition of phenol red and dextrose. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID PRODUCTION
Escherichia coli	25922	10^2-10^3	Good	Positive
Staphylococcus aureus	25923	10^2-10^3	Good	Positive

Formula

Difco™ Peptone Water

Approximate Formula* Per Liter

Peptone	10.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 15 g of the powder in 1 L of purified water.
2. Warm slightly with frequent agitation to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

For Determining Carbohydrate Fermentation Patterns

1. Add 1.8 mL 1% phenol red solution to 1 liter rehydrated Peptone Water. Mix thoroughly.
2. Dispense into test tubes containing inverted Durham vials.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add sufficient sterile carbohydrate solution to yield a 1% final concentration. Rotate each tube to thoroughly distribute the carbohydrate.

Procedure

For Determining Carbohydrate Fermentation Patterns

1. Inoculate tubes with test organisms.
2. Incubate tubes at 35 ± 2°C for 18-48 hours.
3. Observe for color change.

For Performing the Indole Test

1. Inoculate tubes with test organisms.
2. Incubate tubes at 35 ± 2°C for 24 or 48 hours.
3. Using an inoculation loop, spread a loopful of culture over the reaction area of a **BBL™ DrySlide™** Indole slide.
4. Examine the reaction area for appearance of a pink color within 30 seconds.

Expected Results

For Determining Carbohydrate Fermentation Patterns

Acid is produced when carbohydrates are fermented. This is indicated by a yellow color in the medium. Gas production is indicated by the presence of gas bubbles in the fermentation tube.

For Performing the Indole Test

Observe for the formation of a pink color in the DrySlide reaction area, which indicates a positive test for indole production.

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.). 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Peptone Water

ISO

Cat. No. 218071 Dehydrated – 500 g

Europe

Cat. No. 257631 Prepared Bottle, 400 mL

BBL™ DrySlide™ Indole

Cat. No. 231748 Pkg. – 25 x 3 slides

Petragnani Medium

Intended Use

Petragnani Medium is used in qualitative procedures for the isolation and cultivation of mycobacteria from clinical specimens.

Summary and Explanation

Petragnani Medium was described by Norton et al. in their paper regarding culture methods for tubercle bacilli.[1,2]

Petragnani Medium is glycerolated egg medium made with a milk base containing malachite green. Somewhat more inhibitory than ATS and Lowenstein-Jensen media because of higher dye content, Petragnani Medium is particularly recommended for old specimens and for use in parallel with other media for isolation of tubercle bacilli.[2,3]

Principles of the Procedure

The casein peptone, potato flour and skim milk contain amino acids, proteins and carbohydrates necessary for the growth of mycobacteria. The glycerol is a source of energy. Asparagine promotes the initiation of growth and increases the growth rate. Egg yolk is a source of lipids for mycobacterial metabolism. Partial inhibition of bacteria is achieved by the presence of the malachite green dye.

Precaution

Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards.[4]

Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[3] N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. These reagents are provided in the BBL™ MycoPrep™ Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[3,5,6]

Media may be inoculated up to the expiration date and incubated for up to 8 weeks. Following inoculation, keep test containers shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C.

Slanted media should be incubated in a horizontal plane until the inoculum is absorbed. Tubes should have screw caps loose for the first 3 weeks to permit circulation of carbon dioxide for the initiation of growth. Thereafter, to prevent dehydration, tighten caps; loosen briefly once a week. Stand tubes upright if space is a problem.

NOTE: Cultures from skin lesions suspected to contain *M. marinum* or *M. ulcerans* should be incubated at 25-33°C for primary isolation; cultures suspected to contain *M. avium* or *M. xenopi* exhibit optimum growth at 40-42°C.[3] Incubate a duplicate culture at 35-37°C.

Expected Results

Cultures should be read within 5-7 days after incubation and once a week thereafter for up to 8 weeks.

Record Observations.[3]

1. Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7 days. Slow growers require more than 7 days for mature colony forms.
2. Pigment production

   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.

References

1. Norton, Thomas and Broom. 1932. Am. Rev. Tuberc. 25:378.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.
4. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health, 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
5. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Petragnani Medium

Cat. No. 221389 Prepared Slants (C Tubes) – Pkg. of 10*

**Store at 2-8°C.*

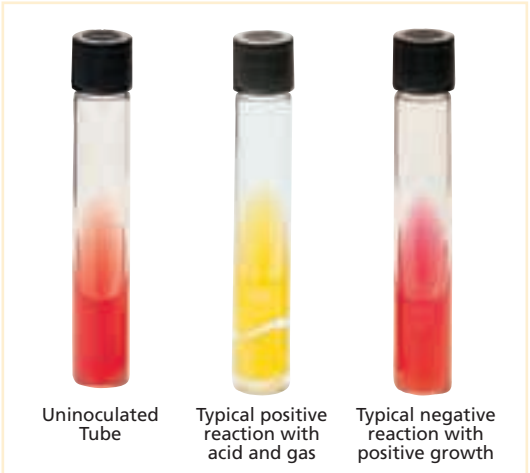
Phenol Red Agar Media
Phenol Red Agar Base • Phenol Red Mannitol Agar

Intended Use
Phenol Red Agar Base is used with added carbohydrate in differentiating pure cultures of bacteria based on fermentation reactions.

Phenol Red Mannitol Agar is used for differentiating pure cultures of bacteria based on mannitol fermentation reactions.

Summary and Explanation
Phenol Red Agar Base with added carbohydrate is well suited for the study of fermentation reactions of microorganisms.[1-4] However, while liquid media are generally employed in studying fermentation reactions, many bacteriologists prefer a solid medium for this purpose. One advantage of a solid fermentation medium is that it permits observation of fermentation reactions under both aerobic and anaerobic conditions. Deep tubes can provide sufficiently anaerobic conditions for the growth of obligate anaerobic bacilli. Any gas formation that occurs during a reaction is indicated by splitting of the agar or accumulation of gas bubbles in the base.

Phenol Red Agar Base supports excellent growth of many fastidious bacteria. It is a basal medium free of any fermentable carbohydrates that could give erroneous interpretations. With the exception of the omitted carbohydrate, it is a complete medium prepared with phenol red as an indicator of reaction changes. Phenol Red Agar Base permits the user to prepare any quantity of medium needed, adding to different portions any fermentable substance to be tested. Usually a final concentration of 0.5-1% of a test carbohydrate is added. An entire series of carbohydrate agars can be made up readily, conveniently and economically. Phenol Red Mannitol Agar already contains the specified carbohydrate.



Uninoculated Tube | Typical positive reaction with acid and gas | Typical negative reaction with positive growth

Principles of the Procedure
Peptone provides the carbon and nitrogen required for good growth of a wide variety of organisms. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. Phenol red serves as a pH indicator, turning from red-orange to yellow when acid is produced during fermentation of the carbohydrate; if the carbohydrate is not fermented, the medium remains red or becomes alkaline (darker red).

Formulae
BBL™ Phenol Red Agar Base
Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Phenol Red	18.0	mg

Difco™ Phenol Red Mannitol Agar
Approximate Formula* Per Liter

Proteose Peptone No. 3	10.0	g
Beef Extract	1.0	g
D-Mannitol	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Phenol Red	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
BBL™ Phenol Red Agar Base
1. Suspend 30 g of the powder in 1 L of purified water. Add carbohydrate, 5-10 g per L if desired. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. If addition of carbohydrate causes a fall in pH, readjust.
3. Dispense and autoclave at 118°C for 15 minutes. Alternatively, sterile carbohydrate solution may be added to cooled autoclaved solution.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Phenol Red Mannitol Agar
1. Suspend 41 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Phenol Red Mannitol Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is orange-red to red, very slightly opalescent.
Prepared Appearance:	Red to orange-red, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Phenol Red Mannitol Agar

Prepare the medium per label directions. Inoculate slant tubes with fresh cultures by stabbing the butt and streaking the slant surface. Incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	ACID	GAS
Escherichia coli	25922	Good	+	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	+	+
Staphylococcus aureus	25923	Good	+	–
Streptococcus mitis	9895	Good	–	–

Identity Specifications

BBL™ Phenol Red Agar Base

Dehydrated Appearance:	Fine, homogeneous, without obvious foreign material.
Solution:	3.0% solution, soluble in purified water upon boiling. Solution is medium to dark, red-orange to rose, clear to slightly hazy.
Prepared Appearance:	Medium to dark, red-orange to rose, clear to slightly hazy.
Reaction of 3.0% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Phenol Red Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 12-18 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Pseudomonas aeruginosa	10145	10^2-10^3	Good

Procedure

1. Inoculate the medium by stabbing into the butt and streaking the surface of the slant. If desired, inoculate obligate anaerobic bacteria into melted medium that has been cooled to 45°C. Allow the agar to solidify prior to incubation.
2. Incubate at 35 ± 2°C for 4-48 hours (or anaerobically for 24-72 hours).
3. Examine periodically for growth, acid production and gas formation.

Expected Results

Fermentation of the carbohydrate is indicated by a change in the color of the medium from red to canary yellow. Gas formation is indicated by the collection of gas bubbles in the base or by splitting of the agar.

For expected reactions with organisms on Phenol Red Agar Base supplemented with various carbohydrates, refer to appropriate references.[1-5]

Limitations of the Procedure

1. The addition of some carbohydrates to the basal medium may cause an acid reaction. To restore the original pH (and color of the medium), add 0.1N sodium hydroxide on a drop-by-drop basis. Take care not to make the medium too alkaline, which would prevent fermentation from occurring within the usual incubation period.
2. When inoculating tubes, stab gently and do not use a loop. Rough stabbing or using a loop to stab may give the false appearance of gas production when mechanical splitting of the medium is what actually occurred.

References

1. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th edition. Mosby, Inc., St. Louis, Mo.
2. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th edition. American Society for Microbiology, Washington, D.C.
3. Holt, Krieg, Sneath, Staley and Williams. 1994. Bergey's Manual™ of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, Md.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition. Elsevier Science Publishing Co., Inc., New York, N.Y.

Availability

BBL™ Phenol Red Agar Base

Cat. No. 211502 Dehydrated – 500 g

Difco™ Phenol Red Mannitol Agar

Cat. No. 210310 Dehydrated – 500 g

Phenol Red Broth Base
Phenol Red Broth with Carbohydrates

Intended Use

Phenol Red Broth Base and Phenol Red Broth with carbohydrates are used for the determination of fermentation reactions in the differentiation of microorganisms.

Summary and Explanation

The ability of an organism to ferment a specific carbohydrate incorporated in a basal medium, resulting in the production of acid or acid and gas, has been used to characterize a specific species or group of bacteria, aid in the differentiation between genera and aid in species differentiation.[1,2]

In 1950, Vera recommended using pancreatic digest of casein in fermentation test media.[3] She found that casein peptone could be used with the pH indicator phenol red in fermentation tests with a high degree of accuracy.

Phenol Red Broth Base and Phenol Red Broth with Carbohydrates are referenced in the *Bacteriological Analytical Manual* for the differentiation of *Bacillus* and *Salmonella*.[4]

Principles of the Procedure

Phenol Red Broth Base is a complete medium without added carbohydrate. It is used as a negative control for fermentation studies or as a base for the addition of carbohydrates by the aseptic addition of **BBL™ Taxo™** Carbohydrate Discs. Pancreatic digest of casein provides nutrients and is low in fermentable carbohydrate.[3] The pH indicator, phenol red, is used to detect acid production.

Phenol Red Broths, prepared with a final concentration of one-half percent carbohydrate, are convenient for the determination of fermentation reactions. Most of the end products

OP

User Quality Control

Identity Specifications

BBL™ Phenol Red Broth Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.5% solution, soluble in purified water. Solution is medium, orange-red to rose-red, clear to slightly hazy.
Prepared Appearance:	Medium, orange-red to rose-red, clear to slightly hazy.
Reaction of 1.5% Solution at 25°C:	pH 7.4 ± 0.2

BBL™ Phenol Red Broth with Dextrose or Lactose or Mannitol or Sucrose

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water. Solution is medium, orange-red to rose-red, clear to slightly hazy.
Prepared Appearance:	Medium, orange-red to rose-red, clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 7.3 ± 0.2 (pH 7.4 ± 0.1 for Sucrose Broth)

Uninoculated Tube | Typical positive reaction with acid and gas | Typical negative reaction with positive growth

Cultural Response

BBL™ Phenol Red Broth Base or Phenol Red Broth with Dextrose or Lactose or Mannitol or Sucrose

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	BASE	DEXTROSE	LACTOSE	MANNITOL	SUCROSE
Escherichia coli	25922	K	AG	AG	AG	
Enterococcus faecalis	33186		A	A		A
Proteus vulgaris	8427	K	A		K	AG
Pseudomonas aeruginosa	10145	K	K			
Salmonella Typhimurium*	14028	K		K		K
Shigella flexneri	9199	K	A	K	A	K
Staphylococcus aureus	25923				A	

**S. enterica* subsp. *enterica* serotype Typhimurium

KEY: A = growth with acid (yellow color)
K = growth with alkaline reaction (red color)
G = gas formation

For quality control organisms for prepared tubes of Phenol Red Broth with the various carbohydrates, consult the ***BBL™ Quality Control and Product Information Manual for Plated and Tubed Media***.[5]

of carbohydrate fermentation are organic acids which, in the presence of phenol red, produce a color change in the medium from red to yellow.[1] If gas is produced during the fermentation reaction, it is collected in the inverted Durham tube.

No yellow color should occur in the control tube. If it does, the results cannot be correctly interpreted since acid has been produced without fermentation.

Formulae

BBL™ Phenol Red Broth Base

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0 g
Sodium Chloride	5.0 g
Phenol Red	18.0 mg

**Adjusted and/or supplemented as required to meet performance criteria.*

BBL™ Phenol Red Carbohydrate Broths

Contain the above ingredients with, per liter, 5.0 g of the specified carbohydrate.

Directions for Preparation from Dehydrated Product

BBL™ Phenol Red Broth Base

1. Dissolve 15 g of the powder in 1 L of purified water.
2. Add carbohydrate (5-10 g), if desired. Agar (0.5-1.0 g) may be added if it is desirable to minimize convection currents in the broth. When agar is added, the medium should be boiled briefly.
3. Dispense in suitable tubes and insert Durham tubes when gas fermentation is to be recorded.
4. Autoclave at 118°C for 15 minutes. Tubes should be packed loosely to ensure free access of steam.
5. Alternatively, the base may be autoclaved and carbohydrates added aseptically as needed.
6. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Phenol Red Broth with Dextrose or Lactose or Mannitol or Sucrose

1. Dissolve 20 g of the powder in 1 L of purified water. Agar (0.5-1.0 g) may be added if it is desirable to minimize convection currents in the broth. When agar is added, the medium should be boiled briefly.
2. Dispense and insert Durham tubes for detection of gas formation.
3. Autoclave at 118°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

If **Taxo** Carbohydrate Discs are being used with tubes of Phenol Red Broth Base, aseptically add the appropriate disc to the tubes prior to inoculation. Using a heavy inoculum, inoculate tubes of media with growth from an 18- to 24-hour pure culture using an inoculating loop. Incubate tubes with loosened caps at 35 ± 2°C for 18-48 hours either in an aerobic or anaerobic atmosphere depending on the organism being evaluated. Incubation up to 30 days may be necessary for a negative result.

Expected Results

Examine the unsupplemented tubes at intervals during the incubation process for growth. If supplemented with carbohydrate, observe for the presence of acid (yellow color) and gas (as evidenced by displacement of the liquid in the Durham tubes).

Consult appropriate references for typical reactions produced by various microbial species.[1,2, 6-8]

References

1. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore, Md.
2. Forbes, Sahm and Weissfeld. 2007. Diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Vera. 1950. Am. J. Public Health, *40*:1267.
4. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
5. Becton, Dickinson and Co. 2007. **BBL**™ quality control and product information manual for plated and tubed media, BD Diagnostics, Sparks, Md.
6. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsvier Science Publishing Co., New York, N.Y.
7. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Phenol Red Broth Base

AOAC BAM CCAM COMPF

Cat. No.	211506	Dehydrated – 500 g
	221897	Prepared Tubes (K Tubes) – Pkg. of 10*

BBL™ Phenol Red Dextrose Broth

BAM

Cat. No.	211514	Dehydrated – 500 g

BBL™ Phenol Red Lactose Broth

BAM

Cat. No.	211519	Dehydrated – 500 g

BBL™ Phenol Red Mannitol Broth

BAM

Cat. No.	211527	Dehydrated – 500 g

BBL™ Phenol Red Sucrose Broth

BAM

Cat. No.	211533	Dehydrated – 500 g

BBL™ Phenol Red Broth with Carbohydrates and Durham Tube

BAM

Cat. No.	221671	Prepared Tubes with Adonitol – Pkg. of 10*
	221673	Prepared Tubes with Arabinose – Pkg. of 10*
	221675	Prepared Tubes with Cellobiose – Pkg. of 10*
	221677	Prepared Tubes with Dextrose – Pkg. of 10*
	221679	Prepared Tubes with Dulcitol – Pkg. of 10*
	221681	Prepared Tubes with Glycerol – Pkg. of 10*
	221683	Prepared Tubes with Inositol – Pkg. of 10*
	221685	Prepared Tubes with Inulin – Pkg. of 10*
	221687	Prepared Tubes with Lactose – Pkg. of 10*
	221689	Prepared Tubes with Maltose – Pkg. of 10*
	221691	Prepared Tubes with Mannitol – Pkg. of 10*
	221693	Prepared Tubes with Raffinose – Pkg. of 10*
	221695	Prepared Tubes with Rhamnose – Pkg. of 10*
	221697	Prepared Tubes with Salicin – Pkg. of 10*
	221699	Prepared Tubes with Sorbitol – Pkg. of 10*
	221701	Prepared Tubes with Sucrose – Pkg. of 10*
	221703	Prepared Tubes with Trehalose – Pkg. of 10*
	221705	Prepared Tubes with Xylose – Pkg. of 10*

**Store at 2-8°C.*

Phenylalanine Agar
Ferric Chloride Reagent

Intended Use

Phenylalanine Agar is used for the differentiation of enteric bacilli on the basis of their ability to produce phenylpyruvic acid by oxidative deamination. Ferric Chloride Reagent is used to visualize the phenylalanine deamination reaction.

Summary and Explanation

Henrickson initially demonstrated that *Proteus* species were able to transform phenylalanine to phenylpyruvic acid.[1] Singer and Volcani,[2] Hamida and LeMinor[3] and others studied the reaction and emphasized its usefulness in the taxonomy of the *Enterobacteriaceae*.

Buttiaux et al. developed a culture medium containing phenylalanine in their study of the characteristic biochemical properties of the *Proteus* and *Providencia* genera.[4] This medium was designed to differentiate members of the *Proteeae* from other members of the *Enterobacteriaceae* by the ability of organisms in the genera within the *Proteeae* to deaminate phenylalanine to phenylpyruvic acid by enzymatic activity.[5] *Proteus*, *Providencia* and *Morganella* species possess this capability. This formula conforms to the modified formula of Ewing et al.[6]

Ferric Chloride Reagent is used to determine if a specific microorganism is capable of producing phenylpyruvic acid from phenylalanine.[5]

Principles of the Procedure

The phenylalanine serves as the substrate for enzymes which are able to deaminate it to form phenylpyruvic acid. The addition of 3-5 drops of a 10% aqueous ferric chloride solution (or a 12% aqueous ferric chloride solution acidified with 2.5 mL of concentrated HCl per 100 mL of reagent) to the cultures following incubation results in the appearance of a light to deep green color (positive reaction) or no color change (negative reaction). In a positive reaction, any phenylpyruvic acid present will react with the ferric salt in the reagent to give a green color.

Formulae

Difco™ Phenylalanine Agar

Approximate Formula* Per Liter

DL-Phenylalanine	2.0	g
Yeast Extract	3.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	1.0	g
Agar	12.0	g

BBL™ Phenylalanine Agar

Approximate Formula* Per Liter

DL-Phenylalanine	2.0	g
Yeast Extract	3.0	g
Sodium Chloride	5.0	g
Sodium Phosphate	1.0	g
Agar	12.0	g

Difco™/BBL™ Ferric Chloride Reagent Droppers

Contain 0.5 mL of 10% ferric chloride in aqueous solution.

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Phenylalanine Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 2.3% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Phenylalanine Agar

Prepare the medium per label directions. Inoculate slant tubes and incubate at 35 ± 2°C for 18-24 hours. After reading for growth, add 3-5 drops of a 10% aqueous solution of ferric chloride and gently rotate to loosen the growth. Read reactions after 1-5 minutes.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR REACTION
Enterobacter aerogenes	13048	10^2-10^3	Good	–
Proteus vulgaris	13315	10^2-10^3	Good	+ (green)
Providencia alcalifaciens	9886	10^2-10^3	Good	+ (green)

Identity Specifications

BBL™ Phenylalanine Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is pale to light, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Pale to light, yellow to tan, slightly hazy.
Reaction of 2.3% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Phenylalanine Agar

Prepare the medium per label directions. Inoculate slant tubes and incubate at 35 ± 2°C for 18-24 hours. After reading growth, add 3-5 drops of a 10% aqueous solution of ferric chloride and gently rotate to loosen the growth. Read reactions after 1-5 minutes.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR REACTION
Escherichia coli	25922	10^2-10^3	Good	–
Proteus vulgaris	8427	10^2-10^3	Good	+ (green)

Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in tubes for slant cultures.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Using a heavy inoculum, inoculate tubed slants with growth from an 18- to 24-hour pure culture. Incubate tubes aerobically at 35 ± 2°C for 4 hours or 18-24 hours. If the inoculum is sufficiently heavy, a 4-hour incubation period should be adequate.[5]

Uninoculated Tube with Reagent

Proteus vulgaris ATCC™ 13315

Expected Results

Following the incubation period, add 3-5 drops of the ferric chloride reagent to the slants. Gently rotate the tube to loosen the growth. Observe for the production of a green color (positive reaction) within 1-5 minutes.

Members of *Proteus*, *Morganella* and *Providencia* genera produce positive results. Most other genera within the *Enterobacteriaceae* are negative for phenylpyruvic acid production.[7,8]

References

1. Henrikson. 1950. J. Bacteriol. *60*:225.
2. Singer and Volcani. 1955. J. Bacteriol. *69*:303.
3. Hamida and LeMinor. 1956. Ann. Inst. Pasteur. *90*:671.
4. Buttiaux, Osteux, Fresnoy and Moriamez. 1954. Ann. Inst. Pasteur Lille. *87*:375.
5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
6. Ewing, Davis and Reavis. 1957. Public Health Lab. *15*:153.
7. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Phenylalanine Agar

BAM

Cat. No. 274520 Dehydrated – 500 g

BBL™ Phenylalanine Agar

BAM

Cat. No. 211537 Dehydrated – 500 g

Difco™/BBL™ Ferric Chloride Reagent (10%)

Cat. No. 261190 Droppers, 0.5 mL – Ctn. of 50

**Store at 2-8°C.*

Phenylethyl Alcohol Agar
Phenylethyl Alcohol Agar with 5% Sheep Blood

Intended Use

Phenylethyl Alcohol (PEA) Agar is a selective medium for the isolation of gram-positive organisms, particularly gram-positive cocci, from specimens of mixed gram-positive and gram-negative flora.[1] The medium, when supplemented with 5% sheep blood, should not be used for determination of hemolytic reactions since atypical reactions may be observed.

Summary and Explanation

After noting that phenylethyl alcohol exhibited an inhibitory effect on gram-negative bacteria with only slight effect on gram-positive organisms, Lilley and Brewer incorporated the chemical in an infusion agar base as a selective agent for the isolation of gram-positive bacteria.[2] Phenylethyl Alcohol Agar, unsupplemented or supplemented with 5% sheep blood, is used in the microbiology laboratory to inhibit gram-negative bacteria, particularly *Proteus*, in specimens containing a mixed bacterial flora.

Principles of the Procedure

Phenylethyl Alcohol Agar and Phenylethyl Alcohol Agar with 5% Sheep Blood support the growth of gram-positive bacterial species, due to the content of peptones, which supply nitrogen, carbon, sulfur and trace nutrients. Sodium chloride maintains osmotic equilibrium. Sheep blood is a source of growth factors. Phenylethyl alcohol is bacteriostatic for gram-negative bacteria since it selectively and reversibly inhibits DNA synthesis.[3]

Formula

BBL™ Phenylethyl Alcohol Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Papaic Digest of Soybean Meal	5.0	g
Sodium Chloride	5.0	g
β-Phenylethyl Alcohol	2.5	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

BBL™ Phenylethyl Alcohol Agar

Dehydrated Appearance:	Slightly moist and softly clumped, resembling "brown sugar" in consistency and appearance.
Solution:	4.25% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 4.25% Solution at 25°C:	pH 7.3 ± 0.2

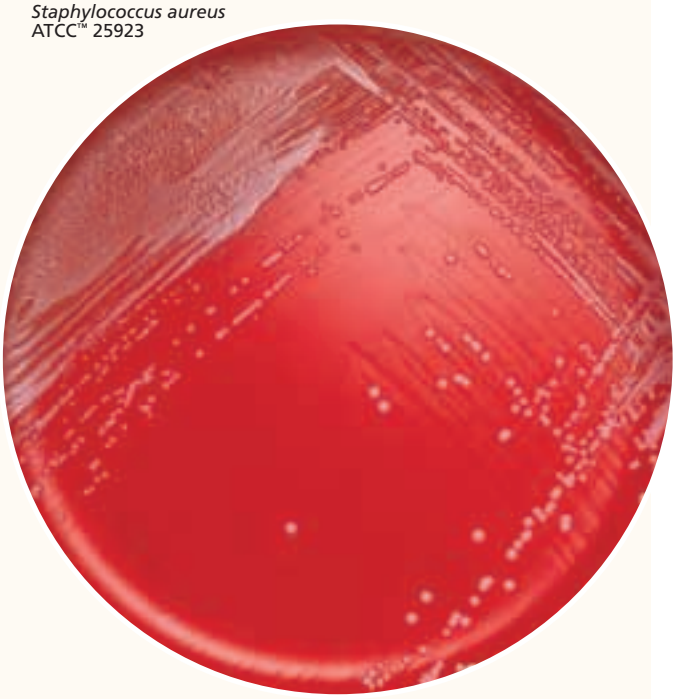
Cultural Response

BBL™ Phenylethyl Alcohol Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C with 3-5% CO_2 for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Proteus mirabilis	12453	10^4-10^5	Partial to complete inhibition
Staphylococcus aureus	25923	10^3-10^4	Good
Streptococcus pneumoniae	6305	10^3-10^4	Good, alpha hemolysis
Streptococcus pyogenes	19615	10^3-10^4	Good, beta hemolysis

Staphylococcus aureus
ATCC™ 25923



OP

Directions for Preparation from Dehydrated Product

1. Suspend 42.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45°C and add 5% sterile defibrinated blood, if desired.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates 24-48 hours at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.

Expected Results

Examine plates for growth of gram-positive organisms.

References

1. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Lilley and Brewer. 1953. J. Am. Pharm. Assoc. *42*:6.
3. Dowell, Hill and Altemeir. 1964. J. Bacteriol. *88*:1811.

Availability

BBL™ Phenylethyl Alcohol Agar

Cat. No. 211539 Dehydrated – 500 g

BBL™ Phenylethyl Alcohol Agar with 5% Sheep Blood

BS12 MCM9

United States and Canada

Cat. No. 221179 Prepared Plates – Pkg. of 20*
221277 Prepared Plates – Ctn. of 100*

Japan

Cat. No. 212086 Prepared Plates – Pkg. of 20*
251277 Prepared Plates – Ctn. of 100*

Mexico

Cat. No. 252569 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Phosphate Buffer, pH 7.2

Intended Use

Phosphate Buffer, pH 7.2 is used for the preparation of dilution blanks for use in the examination of waters, dairy products, foods and other materials.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

The formula for phosphate buffer was originally specified by the American Public Health Association (APHA) for use in diluting test samples. Phosphate Buffer, pH 7.2 still is specified for use in diluting water samples,[4] dairy products[5] and foods[6-9] in standard microbiological methods. In some compendial methods,[6,7] this product is referred to as Butterfield's Phosphate

User Quality Control

Identity Specifications

BBL™ Phosphate Buffer, pH 7.2

Dehydrated Appearance:	White, fine, homogeneous, free of extraneous-material.
Solution:	3.4% solution, soluble in purified water. Solution is colorless, clear to trace hazy.
Prepared Appearance:	Colorless, clear to trace hazy.
Stock Solution at 25°C:	pH 7.2 ± 0.5

BBL™ Phosphate Buffer, pH 7.2 (prepared)

Appearance:	Colorless to light yellow and clear to trace hazy.
Reaction at 25°C:	pH 7.2 ± 0.1

Survival Test

BBL™ Phosphate Buffer, pH 7.2 (prepared, 500 mL bottle, Working Solution)

Perform a 10-minute survival test on the buffer using appropriate dilutions of organisms. In a sterile Petri dish, combine 1 mL of organism dilution with 20 mL of autoclaved and cooled (45-50°C) Standard Methods Agar or Tryptic Soy Agar. Incubate at 35 ± 2°C for up to 3 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AT 10 MINUTES
Bacillus cereus	11778	30-300	≥85% of time zero counts
Candida albicans	10231	30-300	≥85% of time zero counts

BBL™ Phosphate Buffer, pH 7.2 (prepared, 100 mL bottle, Stock Solution)

Perform a 10-minute survival test on a 1:800 dilution of the stock solution using appropriate dilutions of organisms and the membrane filtration test. Incubate organisms for up to 5 days at 30-35°C (incubate *A. brasiliensis* and *C. albicans* at 20-25°C for up to 5 days).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AT 10 MINUTES
Aspergillus brasiliensis (niger)	16404	10-100	>80% of time zero counts
Bacillus subtilis	6633	10-100	>80% of time zero counts
Candida albicans	10231	10-100	>80% of time zero counts
Escherichia coli	8739	10-100	>80% of time zero counts
Pseudomonas aeruginosa	9027	10-100	>80% of time zero counts
Salmonella Abony DSM 4224		10-100	>80% of time zero counts
Staphylococcus aureus	6538	10-100	>80% of time zero counts

Buffered Dilution Water. Some methods[4] require the addition of 5.0 mL of a magnesium chloride solution (81.1 g $MgCL_2 \bullet 6H_2O$ per L of purified water) to the product. General chapters <61> and <62> of the *USP* recommend the use of Phosphate Buffer, pH 7.2 for preparing dilutions of nonsterile pharmaceutical products when performing Microbial Enumeration Tests and Tests for Specified Microorganisms.[1]

Principles of the Procedure

Phosphate buffer is used in the preparation of dilution blanks for use in microbiological testing rather than unbuffered water in order to standardize this potential variable due to the wide variation in the pH of purified water from multiple sources. Sodium carbonate is a pH regulator.

Formula

BBL™ Phosphate Buffer, pH 7.2

Approximate Formula* Per Liter		
Potassium Dihydrogen Phosphate	26.22	g
Sodium Carbonate	7.78	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Prepare a *stock solution*, according to standard procedure, by dissolving 34.0 g in purified water and make up to 1 L.
2. Dispense and sterilize, if desired. Store under refrigeration.
3. Prepare a *working solution* for use in dilution blanks, according to standard procedure, by adding 1.25 mL of *stock solution* to purified water* and make up to 1 L (1:800).
4. Dispense in bottles or tubes to provide a post-autoclaving volume of 99 ± 2 mL or 9 ± 0.2 mL or other appropriate quantity.
5. Autoclave at 121°C for 15 minutes.

*NOTE: If desired, add 5.0 mL of magnesium chloride solution (81.1 g $MgCl_2 \bullet 6 H_2O$ per L of purified water).

Sample Collection and Handling

For water, dairy and food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[4-9]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For water, dairy and food samples, refer to appropriate standard references for details on test methods for using Phosphate Buffer, pH 7.2.[4-9]

For pharmaceutical samples, refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products using Phosphate Buffer, pH 7.2.[1]

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.

3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
5. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. U.S. Food and Drug Administration. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
9. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

BBL™ Phosphate Buffer, pH 7.2

AOAC | BAM | COMPF | EP | JP | SMD | SMWW | USDA | USP

Cat. No.	211544	Dehydrated – 500 g†
	214973	Prepared Bottles (Working Solution), 500 mL (septum screw cap) – Pkg. of 10†
	257385	Prepared Bottles (Stock Solution), 100 mL (septum screw cap) – Ctn. of 25†

† QC testing performed according to USP/EP/JP performance specifications.

OP

Phosphate Buffered Saline

Intended Use

Phosphate Buffered Saline (PBS) is used in cold enrichment procedures to enhance the recovery of *Yersinia enterocolitica*.

Summary and Explanation

Phosphate Buffered Saline is a modified phosphate buffered substrate used in cold enrichment procedures. Cold enrichment procedures utilize the ability of *Y. enterocolitica* to grow at 4°C to improve the recovery of certain serotypes of this bacterium from foods, environmental samples and clinical specimens from asymptomatic carriers and patients who are beyond the acute (diarrheal) stage of illness.[1-7] Direct plating of clinical specimens without cold enrichment is adequate for recovery of *Y. enterocolitica* during the acute stage of illness.

The addition of certain nutrients to Phosphate Buffered Saline is recommended for cold enrichment with meats, vegetable products and other food samples.[8]

Principles of the Procedure

In cold enrichment procedures, stool specimens or other materials are inoculated into the saline solution and refrigerated at 4°C for up to 3 weeks.[2-6] Sodium chloride maintains osmotic equilibrium and potassium phosphate salts buffer the medium to maintain an alkaline pH. *Y. enterocolitica* can survive and grow at the low temperature, while most other microorganisms cannot.

Procedure

Inoculate Phosphate Buffered Saline with approximately 1 g of stool specimen and refrigerate at 2-8°C for up to 3 weeks. Subculture may be done at 3, 7, 14 and, for maximal recovery, 21 days to CIN Agar and/or MacConkey Agar.[1]

Expected Results

Observe for growth of *Yersinia enterocolitica* upon subculture.

References

1. Farmer, Wells, Griffin and Wachsmuth. 1987. *In* Wentworth (ed.), Diagnostic procedures for bacterial infections, 7th ed. American Public Health Association, Washington, D.C.
2. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
3. Greenwood, Flanigan, Pickett and Martin. 1975. J. Clin. Microbiol. 2:559.
4. Eiss. 1975. Scand. J. Infect. Dis. 7:249.
5. Toma and Deidrick. 1975. J. Clin. Microbiol. 2:478.
6. Pai, Sorger, LaFleur, Lackman and Marks. 1979. J. Clin. Microbiol. 9:712.
7. Swaminathan, Harmon and Mehlman. 1982. J. Appl. Bacteriol. *52*:151.
8. Mehlman, Aulisio and Sanders. 1978. J. Assoc. Anal. Chem. *61*:761.

Availability

BBL™ Phosphate Buffered Saline

Cat. No.	297485	Prepared Tubes (K Tubes) – Pkg. of 10

Phytone™ Peptone • Select Phytone™ UF Select Soytone • Bacto™ Soytone

Intended Use

Phytone Peptone, Select **Phytone** UF, Select Soytone and **Bacto** Soytone are used in media for the cultivation of a large variety of organisms, including fungi.

Summary and Explanation

Phytone Peptone is an animal-free soy peptone. **Phytone** Peptone retains the high vitamin and high carbohydrate content of the soy plant tissue. It is an excellent plant peptone for the cultivation of fungi and fastidious types of bacteria, such as members of the *Clostridium* and *Neisseria* genera.[1] It has been used in cell culture applications due to its high carbohydrate content.

Select **Phytone** UF is an ultra-filtered peptone that was developed specifically for the tissue culture market. Its nitrogen content combined with the naturally occurring vitamins has demonstrated remarkable growth support with monoclonal antibodies and protein expression. It has an endotoxin level of less than or equal to 500 EU/g, which makes it an ideal substitute or supplement for fetal bovine serum in cell culture applications.

Select Soytone demonstrates excellent growth support for *Escherichia coli*. Select Soytone is also used in molecular genetics media such as **Select APS**™ Super Broth. Subtle differences in the hydrolysation process give Select Soytone improved performance in cell culture.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

BBL™ Phytone™ Peptone

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 2.0% solution, soluble in purified water. Solution is clear to slightly hazy.

Reaction of 2.0% Solution at 25°C: pH 6.5-7.5

Difco™ Select Phytone™ UF

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 2.0% solution, soluble in purified water. Solution is clear to slightly hazy, no flocculation.

Reaction of 2.0% Solution at 25°C: pH 6.5-7.5

Cultural Response

Biochemical Reactions

BBL™ Phytone™ Peptone

Prepare a sterile solution of **Phytone** Peptone as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	29552	~10^7	Positive
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Citrobacter freundii*	8454	0.1 mL, undiluted	Positive

Growth Response

BBL™ Phytone™ Peptone

Prepare a sterile solution of peptone agar without (plain) and with 5% sheep blood (SB) using 10 g of **Phytone** Peptone, 2.5 g of sodium chloride and 6.5 g of agar in 500 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 2-3 days (incubate streptococci with 3-5% CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Staphylococcus aureus	6538P	10^3-10^4	Good	N/A
Streptococcus pneumoniae	6305	10^3-10^4	N/A	Good, alpha hemolysis
Streptococcus pyogenes	49117	10^4-10^5	Good	Good, beta hemolysis

Continued

Bacto Soytone was found to be effective in the recovery of stressed *E. coli*.[2] It was found that **Bacto** Soytone with the addition of 7 vitamins replaced yeast extract as an economical alternative for the production of lactic acid by *Lactobacillus rhamnosus*.[3] It should be noted that **Bacto** Soytone utilizes an enzyme in the digestion of the soy flour that has been sourced from animal.

Media formulations containing **Phytone** Peptone or **Bacto** Soytone are specified in various standard methods for multiple applications.[4-8]

Principles of the Procedure

Phytone Peptone, Select **Phytone** UF, Select Soytone and **Bacto** Soytone are enzymatic digests of soybean meal. The nitrogen source in the soy peptones contains naturally occurring high concentrations of vitamins and carbohydrates of soybean.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Phytone** Peptone, Select **Phytone** UF, Select Soytone and **Bacto** Soytone in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for specific procedures using **Phytone** Peptone, Select **Phytone** UF, Select Soytone and **Bacto** Soytone.

Identity Specifications

Difco™ Select Soytone

Dehydrated Appearance: Tan, free-flowing, homogeneous powder.

Solution: 2.0% solution, soluble in purified water. Solution is clear to moderately hazy.

Reaction of 2.0% Solution at 25°C: pH 6.5-7.5

Bacto™ Soytone

Dehydrated Appearance: Light to medium tan, free-flowing, homogeneous.

Solution: 2.0% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly hazy. A small amount of precipitate is acceptable.

Reaction of 2.0% Solution at 25°C: pH 6.5-7.5

Cultural Response

Biochemical Reactions

Bacto™ Soytone

Prepare sterile solutions of **Bacto** Soytone as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922	~10^7	Slight positive
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	0.1 mL, undiluted	Positive

Growth Response

Bacto™ Soytone

Prepare a sterile solution with 2% **Bacto** Soytone, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Staphylococcus aureus	6538P	10^3-10^4	Good
Streptococcus pneumoniae	6305	10^3-10^4	Good
Streptococcus pyogenes	49117	10^4-10^5	Good

Expected Results

Refer to appropriate references and procedures for results.

References

1. Power (ed.). 1988. Manual of BBL™ products and laboratory procedures, 6th ed. Becton Dickinson Microbiology Systems, Cockeysville, Md.
2. Chou and Cheng. 2000. Int. J. Food Microbiol. *61*:127.
3. Kwon, Lee, Lee, Chang, Keun and Chang. 2000. Enzyme Microb. Technol. 26:209.
4. Horowitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
5. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
6. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, USEPA, Washington, D.C.
7. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington D.C.
8. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

BBL™ Phytone™ Peptone

AOAC BAM EPA SMWW USDA

Cat. No. 211906 Dehydrated – 454 g
298147 Dehydrated – 5 lb (2.3 kg)
292450 Dehydrated – 10 kg

Difco™ Select Phytone™ UF

Cat. No. 210931 Dehydrated – 500 g
210936 Dehydrated – 10 kg

Difco™ Select Soytone

Cat. No. 212488 Dehydrated – 500 g
212489 Dehydrated – 10 kg

Bacto™ Soytone

AOAC BAM EPA SMWW USDA

Cat. No. 243620 Dehydrated – 500 g
243610 Dehydrated – 10 kg

Phytone™ Yeast Extract Agar

Intended Use

Phytone Yeast Extract Agar is used for the selective isolation of dermatophytes, particularly *Trichophyton verrucosum*, and other pathogenic fungi from routine clinical specimens.

Summary and Explanation

Carmichael and Kraus modified the classical formula of Sabouraud medium in order to selectively recover *Trichophyton verrucosum*, one of the species associated with ringworm, from clinical specimens.[1,2]

Phytone Yeast Extract Agar is used in Petri dishes for early detection of dermatophytes. Skin scrapings or hairs are rubbed over the surface of the agar. Blood agar plates should be inoculated in parallel to permit isolation of pyogenic cocci which may also be present. The medium is of value for increasing the yield of isolation of ringworm organisms and for early identification, especially of *T. verrucosum*.

Principles of the Procedure

The incorporation of the antimicrobial agents streptomycin and chloramphenicol in the medium inhibits growth of bacterial species and promotes detection of pathogenic fungi.

User Quality Control

Identity Specifications

BBL™ Phytone™ Yeast Extract Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	7.2% solution, soluble in purified water upon boiling. Solution is light to dark, yellow to tan, clear to moderately hazy.
Prepared Appearance:	Light to dark, yellow to tan, clear to moderately hazy.
Reaction of 7.2% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

BBL™ Phytone™ Yeast Extract Agar

Prepare the medium per label directions. Inoculate with fresh cultures as described below and incubate at 25 ± 2°C for 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	Undiluted	Good
Candida albicans	10231	Undiluted	Good
Penicillium roquefortii	9295	Undiluted	Good
Staphylococcus aureus	25923	10^4-10^5	Complete inhibition
Trichophyton verrucosum	38485	Undiluted	Good

Formula

BBL™ Phytone™ Yeast Extract Agar

Approximate Formula* Per Liter

Papaic Digest of Soybean Meal	10.0	g
Yeast Extract	5.0	g
Dextrose	40.0	g
Streptomycin	0.03	g
Chloramphenicol	0.05	g
Agar	17.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 72 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate skin scrapings, hair or other materials directly on the agar surface of Petri plates. Incubate plates in an aerobic atmosphere at 25-30°C or at 30-37°C if *T. verrucosum* is suspected.

Expected Results

After the plates have been incubated for 2-3 days, examine them directly under the microscope. If microcolonies are observed, they should be transferred to fresh plates before the original plates become overgrown.

References

1. Carmichael and Kraus. 1959. Alberta Med. Bull. *24*:201.
2. Carmichael. 1961. Mycopathologia *14*:129.

Availability

BBL™ Phytone™ Yeast Extract Agar

Cat. No. 211546 Dehydrated – 500 g

Plate Count Agar/Standard Methods Agar (Tryptone Glucose Yeast Agar)

Intended Use

Plate Count Agar and Standard Methods Agar (Plate Count Agar; Tryptone Glucose Yeast Agar) are used for obtaining microbial plate counts from milk and dairy products, foods, water and other materials of sanitary importance.

Summary and Explanation

Plate Count Agar and Standard Methods Agar are made according to the American Public Health Association (APHA) formulation.[1] They are recommended for obtaining plate counts for milk and other dairy products and may also be used to determine the sanitary quality of foods, water and other materials.[1-5]

Each lot of dehydrated medium base is subjected to the APHA quality control test and has met the APHA requirements.[1,6] Appropriate references should be consulted for standard plate count procedures recommended by the APHA and other agencies.[1-5]

The **Hycheck**™ hygiene contact slide is a double-sided paddle containing two agar surfaces for immersing into fluids or sampling surfaces. There are two slides with Plate Count Agar: one contains Plate Count Agar on one side of the slide and the medium with triphenyltetrazolium chloride (TTC) on the other side; the second slide contains Plate Count Agar with TTC on both sides.

Principles of the Procedure

Enzymatic digest of casein provides the amino acids and other complex nitrogenous substances necessary to support bacterial growth. Yeast extract primarily supplies the B-complex vitamins, and dextrose is an energy source. TTC is reduced to the insoluble formazan inside the bacterial cell producing red-colored colonies.

Formula

Difco™ Plate Count Agar or BBL™ Standard Methods Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Yeast Extract	2.5	g
Dextrose	1.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 23.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for information regarding the processing and inoculation of food, water samples and other materials.[1-5]

Liquefy the medium in pour tubes and bottles by heating in boiling water. Cool to 45-50°C.

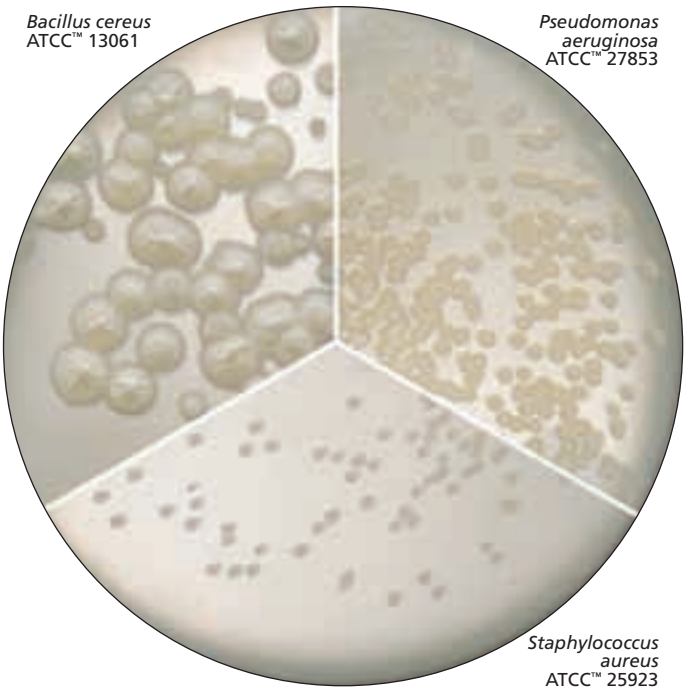
Usually 1 mL samples of appropriate dilutions of the test sample are pipetted into sterile Petri dishes and molten, cooled medium is added followed by gently mixing to distribute the sample dilution throughout the agar. Incubate hardened plates for 48 ± 3 hours at 32 ± 1°C (dairy products) or 35 ± 0.5°C (foods) in an aerobic atmosphere.

Expected Results

Follow recommended procedures for the counting of colonies and the reporting of results.[1-5]

References

1. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
4. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
5. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
6. Marth (ed.). 1978. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.



User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Plate Count Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.35% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 2.35% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Plate Count Agar

Prepare the medium per label directions. Inoculate using the pour plate method and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus johnsonii	11506	30-300	Good
Staphylococcus aureus	25923	30-300	Good

Identity Specifications

BBL™ Standard Methods Agar

Dehydrated Appearance:	Fine to medium fine, may contain small tan and white flecks, homogeneous, free of extraneous material.
Solution:	2.35% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly opalescent.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly opalescent.
Reaction of 2.35% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

BBL™ Standard Methods Agar

Prepare the medium per label directions. Inoculate using the pour plate method and incubate *Bacillus stearothermophilus* at 55-60°C and 35 ± 2°C for all other organisms for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	30-300	Good
Bacillus stearothermophilus	7953	30-300	Good
Enterococcus hirae	10541	30-300	Good
Escherichia coli	25922	30-300	Good
Lactobacillus rhamnosus	7469	30-300	Good
Lactobacillus delbruecki subsp. *lactis*	12315	30-300	Good

Availability

Difco™ Plate Count Agar

AOAC | BAM | CCAM | COMPF | EPA | ISO | SMD | SMWW | USDA

Cat. No. 247930 Dehydrated – 100 g
247940 Dehydrated – 500 g
247910 Dehydrated – 2 kg
247920 Dehydrated – 10 kg

Difco™ Hycheck™ Hygiene Contact Slides

Cat. No. 290005 Plate Count Agar // Plate Count Agar with TTC – Pkg. of 10 slides*
290004 Plate Count Agar with TTC // Plate Count Agar with TTC – Pkg. of 10 slides*

BBL™ Standard Methods Agar

AOAC | BAM | CCAM | COMPF | EPA | ISO | SMD | SMWW | USDA

Cat. No. 212455 Dehydrated – 100 g
211638 Dehydrated – 500 g
211641 Dehydrated – 5 lb (2.3 kg)

United States and Canada

Cat. No. 297030 Prepared Plates – Pkg. of 20*
221030 Prepared Pour Tubes – Pkg. of 10
299094 Prepared Bottles – 10 × 200 mL
299102 Prepared Bottles – 10 × 500 mL

Europe

Cat. No. 254483 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251536 Prepared Plates – Pkg. of 20*
251543 Prepared Plates – Ctn. of 100*
251546 Prepared Plates (150 × 15 mm-style) – Pkg. of 24*
251506 Prepared **RODAC**™ Plates – Pkg. of 30*

Mexico

Cat. No. 252634 Prepared Bottles, 140 mL – Pkg. of 12

**Store at 2-8°C.*

m Plate Count Broth

Intended Use

m Plate Count Broth is used for enumerating microorganisms by membrane filtration.

Summary and Explanation

m Plate Count Broth is a nonselective general-purpose medium for determining bacterial counts from food and water samples using the membrane filtration procedure. Also known as m Tryptone Glucose Yeast Broth or m Standard Methods Broth, this medium has the same formulation as Plate Count Agar except that agar has been omitted and the ingredients are employed in twice the concentration as in the solid medium.[1]

Principles of the Procedure

Yeast extract is a source of trace elements, vitamins and amino acids. Peptone provides carbon and nitrogen for bacterial metabolism. Dextrose is a fermentable carbohydrate and carbon source.

Formula

Difco™ m Plate Count Broth

Approximate Formula* Per Liter

Yeast Extract	5.0	g
Tryptone	10.0	g
Dextrose	2.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 17 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Collect samples according to recommended guidelines.[1,2]
2. Place a sterile absorbent pad in each 50 × 9 mm Petri dish.
3. Saturate the pad with approximately 2.0-2.4 mL of prepared medium.
4. Place an inoculated membrane filter, inoculated side up, on the saturated pad.
5. Incubate in a 35 ± 2°C incubator for 18-24 hours.

Expected Results

After incubation, count the colonies on the surface of the filter. The colonies can be subcultured to appropriate media for identification, if desired.

References

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

User Quality Control

Identity Specifications

Difco™ m Plate Count Broth

Dehydrated Appearance:	Light beige to beige, free-flowing, homogeneous.
Solution:	1.7% solution, soluble in purified water upon warming. Solution is light to medium amber, clear to slightly opalescent, may have a very slight precipitate.
Prepared Appearance:	Light to medium amber, clear to slightly opalescent, may have a very slight precipitate.
Reaction of 1.7% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ m Plate Count Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	19433	10^2-3×10^2	Good
Escherichia coli	25922	10^2-3×10^2	Good
Staphylococcus aureus	25923	10^2-3×10^2	Good
Staphylococcus epidermidis	12228	10^2-3×10^2	Good

Availability

Difco™ m Plate Count Broth

Cat. No. 275120 Dehydrated – 500 g

Polypeptone™ Peptone

Intended Use

Polypeptone Peptone is used as a component in microbiological culture media.

Summary and Explanation

Researchers have found that **Polypeptone** Peptone meets the nutritional requirements of various bacteria, fungi and mammalian cells, where a single source of casein meat peptone has been unsatisfactory. **Polypeptone** Peptone has been utilized in culture media for the production of trypsin inhibitor by *Cephalosporium* sp.,[1] in the production of bacterial cellulose by *Acetobacter* sp. A9[2] and in the production of succinic acid from whey by *Anaerobiospirillum succiniciproducens*.[3] In addition, **Polypeptone** Peptone has been used in the mass production of luciferase-bacterial magnetic particles by recombinant *Magnetospirillum magneticum* AMB-1[4] and the production of a novel tumor-killing factor by human macrophage-monocyte hybridomas.[5]

Media formulations containing **Polypeptone** Peptone are specified in standard methods for various applications.[6-11]

Principles of the Procedure

Polypeptone Peptone is a mixture of peptones made up of equal parts of pancreatic digest of casein and peptic digest of animal tissue. **Polypeptone** Peptone includes the high content of amino acids and small polypeptides characteristic of pancreatic digest of casein and the larger polypeptides characteristic of peptic digest of animal tissue. **Polypeptone** Peptone provides nitrogen, amino acids and vitamins in microbiological culture media.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Polypeptone** Peptone in the formula of the medium being prepared. Add product as required.

Procedure

See appropriate references for specific procedures using **Polypeptone** Peptone.

User Quality Control

Identity Specifications

BBL™ Polypeptone™ Peptone

Dehydrated Appearance:	Light to dark, yellow to tan, fine, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water. Solution is light to medium, yellow to tan, clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 6.8-7.5

Cultural Response

BBL™ Polypeptone™ Peptone

Prepare a sterile solution of peptone agar without (plain) and with 5% sheep blood (SB) using 10 g of **Polypeptone** Peptone, 2.5 g of sodium chloride and 6.5 g of agar in 500 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 2-3 days (incubate streptococci with 3-5% CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Salmonella enterica subsp. *enterica* serotype Typhi	19430	10^3-10^4	Good	N/A
Staphylococcus aureus	6538P	10^3-10^4	Good	N/A
Streptococcus pneumoniae	6305	10^3-10^4	N/A	Good, alpha hemolysis
Streptococcus pyogenes	49117	10^4-10^5	Good	Good, beta hemolysis

Expected Results

Refer to appropriate references and procedures for results.

References

1. Tsuchiya and Kimura. 1978. Appl. Environ. Microbiol. *35*:631.
2. Son, Heo, Kim and Lee. 2001. Biotechnol. Appl. Biochem. *33(Pt 1)*:1.
3. Lee, Lee, Kwon, Lee and Chang. 2000. Appl. Microbiol. Biotechnol. *54*:23.
4. Yang, Takeyama, Tanaka and Matsunaga. 2001. Enzyme Microbiol. Technol. *29*:13.
5. Taniyama, Yoshida and Furuta. 1988. J. Immunol. *141*:4061.
6. Horowitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
7. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
9. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
10. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
11. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

BBL™ Polypeptone™ Peptone

AOAC BAM COMPF SMWW USDA USP

Cat. No.	211910	Dehydrated – 454 g
	297108	Dehydrated – 10 kg

Potato Dextrose Agar • Potato Dextrose Broth

Intended Use

Potato Dextrose Agar is used for the cultivation and enumeration of yeasts and molds.

Potato Dextrose Broth is used for cultivating yeasts and molds.

Potato Dextrose Agar meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Potato Dextrose Agar is a general purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is used in plate count methods when testing food,[4-6] dairy products[7] and cosmetics.[5,6] The *USP* lists Potato Dextrose Agar as one of the recommended media for use in the Microbial Enumeration Tests when testing nonsterile pharmaceutical products.[1]

Potato Dextrose Agar can be used to grow clinically significant yeasts and molds.[8,9] In addition, this medium is used to stimulate sporulation (slide preparations), maintain stock cultures of certain dermatophytes and differentiate atypical varieties of dermatophytes by pigment production.[10]

Potato Dextrose Broth is a general-purpose broth medium for yeasts and molds (Potato Dextrose Agar without the agar).

Principles of the Procedure

Potato starch, potato infusion and dextrose support luxuriant growth of fungi. Lowering the pH of the medium to approximately 3.5 with sterile tartaric acid achieves the inhibition of bacterial growth. It is important, however, to avoid heating the medium after it has been acidified because this action results in the hydrolysis of the agar and impairs its ability to solidify.

Formulae

Difco™ Potato Dextrose Agar

Approximate Formula* Per Liter

Potato Starch (from infusion)**	4.0	g
Dextrose	20.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*
***Approximates 200 g of infusion from potatoes.*

Difco™ Potato Dextrose Broth

Consists of the same ingredients without the agar.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Potato Dextrose Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous (may contain small dark particles).
Solution:	3.9% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 3.9% Solution at 25°C:	pH 5.6 ± 0.2

Difco™ Potato Dextrose Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in purified water upon boiling. Solution is very, very light amber, clear to very slightly opalescent.
Prepared Appearance:	Very, very light amber, clear to very slightly opalescent.
Reaction of 2.4% Solution at 25°C:	pH 5.1 ± 0.2

Cultural Response

Difco™ Potato Dextrose Agar

Prepare the medium per label directions. Inoculate and incubate at 25-30°C for 18-48 hours (up to 7 days for *T. mentagrophytes*). For *Aspergillus brasiliensis*, incubate at 20-25°C for 5 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Candida albicans	10231	10^3-10^4	Good
Saccharomyces cerevisiae	9763	10^3-10^4	Good
Trichophyton mentagrophytes	9533	Undiluted	Good
Aspergillus brasiliensis (niger)	16404	<100	Growth

Difco™ Potato Dextrose Broth

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	10231	30-300	Good
Lactobacillus casei	7469	30-300	Fair to good
Saccharomyces cerevisiae	9763	30-300	Good

Identity Specifications

BBL™ Potato Dextrose Agar (prepared)

Appearance:	Light to medium tan cream and trace hazy.
Reaction at 25°C:	pH 5.6 ± 0.2

Cultural Response

BBL™ Potato Dextrose Agar (prepared)

Inoculate and incubate at 20-25°C for up to 7 days (up to 3 days for *C. albicans*) under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Candida albicans	10231	10-100	Good
Candida albicans	60193	10-100	Good
Saccharomyces cerevisiae	9763	10^3-10^4	Good
Trichophyton mentagrophytes	9533	Undiluted	Good
Aspergillus brasiliensis (niger)	16404	<100	Growth

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Potato Dextrose Agar – 39 g;
   Difco™ Potato Dextrose Broth – 24 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. To alter the reaction of the agar medium to pH 3.5, cool the base to 45-50°C and aseptically add an appropriate amount of sterile 10% tartaric acid to each liter of medium. Mix well. Do not reheat the medium.
5. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[8,9]

For food, dairy and cosmetic samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[4-7]

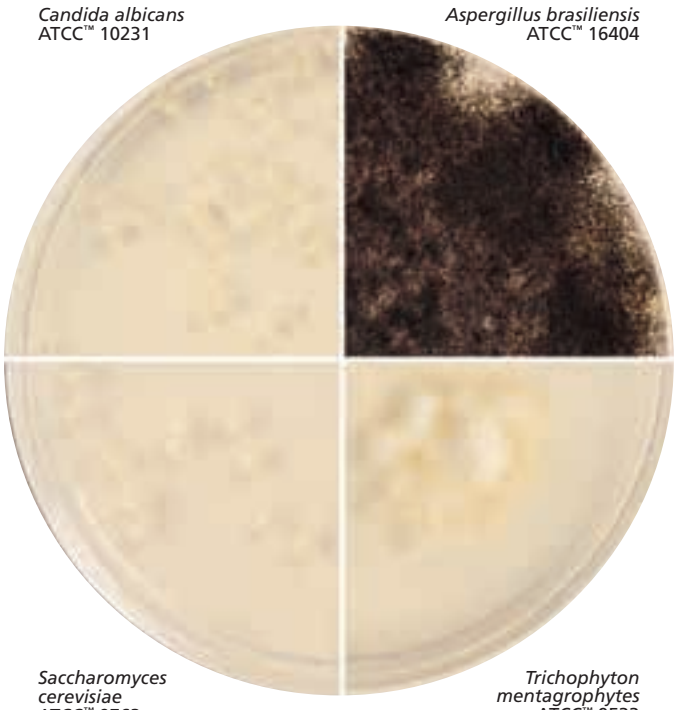
For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using Potato Dextrose Agar.[8,9]

For food, dairy and cosmetic samples, refer to appropriate standard references for details on test methods using Potato Dextrose Agar.[4-7]

For pharmaceutical samples, refer to *USP* General Chapter <61> for details on the examination of nonsterile products and Microbial Enumeration Tests using Potato Dextrose Agar.[1]



Liquefy the medium in pour tubes by heating in boiling water. Cool to 45-50°C and pour into sterile Petri dishes. Allow to solidify for a minimum of 30 minutes.

Streak the specimen onto prepared media with a sterile inoculating loop to obtain isolated colonies. When used for determining yeast and mold counts, the medium should be adjusted to a pH of approximately 3.5 with sterile tartaric acid and used in the standard pour plate technique. Incubate the plates at 25-30°C with increased humidity for up to 7 days.

Tubed slants are used primarily for the cultivation and maintenance of pure cultures. They should be inoculated with an inoculating loop and incubated under the same conditions as the plated medium.

For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Inoculation of Potato Dextrose Broth with pure cultures of yeasts can assist in their identification.

Expected Results

After sufficient incubation, the plates which were streak inoculated should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. The colonies in pour plates should be counted and the results expressed as yeast and mold counts per gram or milliliter of material, taking into account the applicable dilution factor.

Growth from tubes inoculated with pure cultures may be used for biochemical and/or serological testing.

For broth, observe cultures for surface growth and pellicle formation.

Limitations of the Procedure

1. Heating Potato Dextrose Agar after acidifying hydrolyzes the agar and may destroy the solidifying properties.
2. Potato Dextrose Agar is not a differential medium. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
6. U.S. Food and Drug Administration. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
7. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
9. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
10. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Potato Dextrose Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP JP MCM9 SMD USP

Cat. No.	213300	Dehydrated – 100 g†
	213400	Dehydrated – 500 g†
	213200	Dehydrated – 2 kg†

BBL™ Potato Dextrose Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP JP MCM9 SMD USP

Cat. No.	221002	Prepared Pour Tubes, 20 mL – Pkg. of 10
	297241	Prepared Slants – Pkg. of 10
	299906	Prepared Bottles,500 mL (septum screw cap) – Pkg. of 10†

United States and Canada

Cat. No.	296272	Prepared Plates (Deep Fill) – Pkg. of 20*
	297945	Prepared Plates (Deep Fill) – Ctn. of 100*

Japan

Cat. No.	251545	Prepared Plates – Ctn. of 100*
	251821	Prepared Plates (Deep Fill) – Ctn. of 100*
	251544	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*

Mexico

Cat. No.	252632	Prepared Bottles, 140 mL – Pkg. of 12

NOTE: None of the prepared media contain tartaric acid.

Difco™ Potato Dextrose Broth

Cat. No.	254920	Dehydrated – 500 g

** Store at 2-8°C.*
† QC testing performed according to USP/EP/JP performance specifications.

Potato Flakes Agar • Potato Flakes CC Agar Potato Flakes Agar with Chloramphenicol and Gentamicin

Intended Use
These media are used in qualitative procedures for the cultivation of pathogenic and opportunistic fungi encountered in clinical mycology.

Summary and Explanation
Potato Flakes Agar induces sporulation, enhancing the production of morphological structures required for the identification of many pathogenic and opportunistic fungi.[1] The addition of chloramphenicol and cycloheximide (CC) or gentamicin provides selectivity for more effective isolation and identification of medically significant fungi.

Principles of the Procedure
The medium stimulates the production of morphological features, such as conidia configurations, improving the ability to identify fungi by their particular morphological structures. Correct identification of fungi causing human disease depends upon visualization of characteristic morphological features.

The antimicrobial agents chloramphenicol, cycloheximide and gentamicin are incorporated in various combinations to improve the recovery of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.[2] Chloramphenicol is a broad-spectrum antibiotic that inhibits a wide range of gram-positive and gram-negative bacteria. Cycloheximide is an anti-fungal agent that inhibits saprophytic fungi, while permitting the growth of pathogenic species. Gentamicin is an aminoglycoside antibiotic that inhibits growth of gram-negative bacteria.

Procedure
Consult appropriate references for information about the processing and inoculation of specimens such as tissues, skin scrapings, hair, nail clippings, etc.[3-5]

For isolation of fungi causing cutaneous mycoses, a nonselective medium should be inoculated along with a selective medium. Incubate the plates at 25-30°C in an inverted position (agar side up) with increased humidity. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C.

All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Expected Results
Examine the media for growth. Microscopic examination of the colony aids in identification.[6]

References

1. Rinaldi. 1982. J. Clin. Microbiol. *15*:1159.
2. Merz and Roberts. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
4. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
5. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.
6. Larone. 1995. Medically important fungi: a guide to identification, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability
BBL™ Potato Flakes Agar

BS12 CMPH2 MCM9

Cat. No. 298328 Prepared Plates – Pkg. of 10*

BBL™ Potato Flakes CC Agar

Cat. No. 298327 Prepared Plates – Pkg. of 10*

BBL™ Potato Flakes Agar with Chloramphenicol and Gentamicin

Cat. No. 292259 Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

Potato Infusion Agar

Intended Use
Potato Infusion Agar is used for cultivating *Brucella*, especially in mass cultivation procedures.

Summary and Explanation
Potato Infusion Agar is prepared according to the formula used by Stockman and MacFadyean for the isolation of *Brucella abortus*. Brucellosis is a zoonotic disease with a domestic-animal reservoir.[1] Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[1]

Brucella spp. grow on most standard laboratory media, including blood agar and chocolate agar, when incubated at 35°C in a CO_2-supplemented atmosphere; however, enriched media are preferred.[2] Potato Infusion Agar, enriched with glycerol, permits luxuriant growth of characteristic colonies of *B. abortus* from infected materials, and may be used with excellent results in mass cultivation of *Brucella* in the preparation of vaccines and antigens.

Principles of the Procedure

Infusion from potatoes, beef extract and peptone provide the nitrogen, vitamins and amino acids in Potato Infusion Agar. Dextrose and glycerol are used as a carbon source in this formula. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula

Difco™ Potato Infusion Agar

Approximate Formula* Per Liter		
Potatoes, Infusion from 200 g	4.0	g
Beef Extract	5.0	g
Proteose Peptone	10.0	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Potato Infusion Agar

Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	4.9% solution, soluble in purified water with 2% glycerol upon boiling. Solution is medium amber, slightly opalescent, with a slight precipitate.
Prepared Appearance:	Medium amber, slightly opalescent to opalescent with a slight precipitate.
Reaction of 4.9% Solution with 2% Glycerol at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Potato Infusion Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 40-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella abortus	4315*	10^2-10^3	Good
Brucella melitensis	4309*	10^2-10^3	Good
Brucella suis	4314*	10^2-10^3	Good
Streptococcus pneumoniae	6305	30-300	Good

**Minimally, one strain of* Brucella *should be used for performance testing. These ATCC strains should be used if available.*

Precautions[3]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

1. Suspend 49 g of the powder in 1 L of purified water containing 2% glycerol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. The final medium will contain a slight, rapidly-settling precipitate that will not interfere with product performance.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Incubate plates at 35 ± 2°C in 5-10% CO_2 for 10 days.[1] For a complete discussion on the inoculation and identification of *Brucella* spp., consult appropriate references.[1,2]

Expected Results

Refer to appropriate references and procedures for results.

References

1. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Difco™ Potato Infusion Agar

Cat. No. 251100 Dehydrated – 500 g

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

Presence-Absence Broth

Intended Use

Presence-Absence Broth is used for detecting coliforms in treated water.

Summary and Explanation

The Presence-Absence (P-A) test is a presumptive detection test for coliforms in water. The test is a simple modification of the multiple-tube procedure.[1] One test sample, 100 mL, is inoculated into a single culture bottle to obtain qualitative information on the presence or absence of coliforms based on the presence or absence of lactose fermentation.[1] This test is based on the principle that coliforms and other pollution indicator organisms should not be present in a 100 mL water sample.[2-8]

Comparative studies with the membrane filter procedure indicate that the P-A test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection.[1] The P-A test is described in standard methods for water testing[1] and by U.S. Environmental Protection Agency.[9]

User Quality Control

Identity Specifications

Difco™ Presence-Absence Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.05% solution, soluble in purified water. Solution is purple, clear to very slightly opalescent.
Prepared Appearance:	Purple, clear to very slightly opalescent, without significant precipitate.
Reaction of 3.05% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Presence-Absence Broth

Prepare Presence-Absence Broth in triple strength solution (9.15%). Sterilize in 50 mL quantities in milk dilution bottles with capacity greater than 150 mL. Add 100 mL of drinking water after medium is sterilized and cooled to room temperature. Inoculate bottles with the test organisms. Incubate bottles at 35 ± 0.5°C for 18-48 hours.

Presence-Absence Broth

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR CHANGE
Enterococcus faecalis	29212	10^2-10^3	Partial inhibition to fair	No change to slight yellow
Escherichia coli	25922	10^2-10^3	Good	Yellow with or without gas production
Escherichia coli	13762	10^2-10^3	Good	Yellow with or without gas production
Pseudomonas aeruginosa	27853	10^2-10^3	Partial inhibition to fair	No change

Principles of the Procedure

Beef extract and peptones provide the nitrogen, vitamins and amino acids in Presence-Absence Broth. Lactose is the carbon source in the formula. The potassium phosphates provide buffering capacity; sodium chloride provides essential ions. Sodium lauryl sulfate is the selective agent, inhibiting many organisms except coliforms. Bromcresol purple is used as an indicator dye; lactose-fermenting organisms turn the medium from purple to yellow with or without gas production.

Formula

Difco™ Presence-Absence Broth

Approximate Formula* Per Liter

Beef Extract	3.0 g
Peptone	5.0 g
Lactose	7.46 g
Pancreatic Digest of Casein	5.90 g
Proteose Peptone No. 3	3.93 g
Dipotassium Phosphate	1.35 g
Monopotassium Phosphate	1.35 g
Sodium Chloride	2.46 g
Sodium Lauryl Sulfate	0.05 g
Bromcresol Purple	8.5 mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 91.5 g of the powder in 1 L of purified water to prepare at triple strength. Mix thoroughly.
2. Warm gently to completely dissolve the powder.
3. Dispense 50 mL amounts into screw cap 250 mL milk dilution bottles.
4. Autoclave at 121°C for 12 minutes, with total autoclave time not to exceed 30 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Collect water samples as described in recommended procedures.[1,9]
2. Inoculate 50 mL of the sterile triple strength P-A Broth with 100 mL of the water sample.
3. Invert the bottle a few times to achieve an even distribution of the medium throughout the test sample.
4. Incubate at 35 ± 0.5°C.
5. Inspect for acid and gas production after 24 and 48 hours of incubation.

Expected Results

A distinct yellow color indicates lactose fermentation, an acid reaction. Gas production can be observed by a foaming reaction when the bottle is gently shaken. Any amount of gas and/or acid is a positive presumptive test requiring confirmation.[1] Report results as positive or negative for coliforms per 100 mL of sample.

Confirmation and differentiation of coliforms detected by the P-A test may be achieved by use of appropriate confirmatory media, incubation times and temperatures as outlined in appropriate references.[1,9]

Limitations of the Procedure

1. The P-A test is only a presumptive test for coliforms.
2. Extending the P-A test incubation period to 72 or 96 hours will allow isolation of other indicator organisms. However, indicator bacteria isolated after 48 hours incubation may not be considered for regulatory purposes.

References

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Availability

Difco™ Presence-Absence Broth

CCAM EPA SMWW

Cat. No.	219200	Dehydrated – 500 g
	219100	Dehydrated – 2 kg

Bacto™ Proteose Peptone • BiTek™ Proteose Peptone Bacto™ Proteose Peptone No. 2 • Bacto™ Proteose Peptone No. 3 • Bacto™ Proteose Peptone No. 4

Intended Use

Bacto Proteose Peptone, **BiTek** Proteose Peptone, **Bacto** Proteose Peptone No. 2 and **Bacto** Proteose Peptone No. 4 are used in preparing microbiological culture media and in producing bacterial toxins.

Bacto Proteose Peptone No. 3 is used in preparing microbiological culture media.

Summary and Explanation

Studies of peptic digests of animal tissue prepared under varying digestion parameters led to the development of **Bacto** Proteose Peptone, **Bacto** Proteose Peptone No. 2 and **Bacto** Proteose Peptone No. 3. Data accumulated during these studies demonstrated that no one peptone is the most suitable nitrogen source for every microbiological application.

Bacto Proteose Peptone was originally developed to produce a diphtheria toxin of high and uniform potency from cultures of *Corynebacterium diphtheriae*. Studies support the use of **Bacto** Proteose Peptone for production of diphtheria toxin, toxin-antitoxin mixtures and toxoid.[1,2] **Bacto** Proteose Peptone is also valuable in the production of other bacterial toxins such as *Clostridium botulinum* toxin,[3] toxin from *Clostridium perfringens*,[4] toxin of hemolytic streptococci,[5] pneumococcus toxin[6] and toxin from *Salmonella pullorum*.[7]

Many factors account for the suitability of **Bacto** Proteose Peptone for the culture of fastidious pathogens, including the nitrogen components, buffering range and the high content of proteoses. These elements create an environment beneficial to the maintenance of virulence and the elaboration of bacterial by-products. Consequently, stock cultures are well preserved on media containing **Bacto** Proteose Peptone. **Bacto** Proteose Peptone may be used in culture medium for a variety of applications, including production of substances from the culture of bacteria, fungi and mammalian cells. **Bacto** Proteose Peptone has been utilized in a medium for producing glycosidases from *Bacteroides fragilis*[8] and to stimulate amyloglucosidase production by *Aspergillus* sp.[9] It has been used to cultivate halophilic bacteria isolated from soil in Egypt for production of polymers.[10] Jan et al.[11] reported that Proteose Peptone as supplementation to a defined medium resulted in significant increases in cell number and specific monoclonal antibody production in a batch culture system. Proteose Peptone has also been used to provide nutrients for axenic culture of amoeba.[12] Consult standard methods for additional media formulations containing Proteose Peptone.[13-16]

BiTek Proteose Peptone was developed to provide a product priced for the biotechnology/pharmaceutical market with growth characteristics to match **Bacto** Proteose Peptone.

Bacto Proteose Peptone No. 2 was originally developed for use in media for the production of diphtheria toxin. Bunney and Thomas[17] reported good yield of diphtheria toxin with Proteose Peptone No. 2 in a simple peptone-sugar-sodium acetate medium.

Bacto Proteose Peptone No. 3 is a modification of **Bacto** Proteose Peptone adapted for use in the preparation of chocolate agar for propagation of *Neisseria* species and chocolate tellurite agar for *Corynebacterium diphtheriae*. While investigating the nutritional values of the **Bacto** Proteose Peptones, Difco Laboratories found that **Bacto** Proteose Peptone No. 3 provides superior nutrition for fastidious microorganisms. It supports growth of streptococci, staphylococci, pneumococci, gonococci and other organisms that require a highly nutritious substrate. For example, Ifediba and Vanderberg[18] report that Proteose Peptone No. 3 or Neopeptone (both being peptones of meat origin) in addition to calf serum, was used as an inexpensive replacement for human serum in cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Recently, because of the benefit of lower endotoxin levels, cell culture manufacturers have found significant yield improvements in using **Bacto** Proteose Peptone No. 3. Consult standard methods for additional media formulations containing Proteose Peptone No. 3.[14-16,19]

Bacto Proteose Peptone No. 4 is a spray-dried version of **Bacto** Proteose Peptone. It offers the same beneficial nutrients as **Bacto** Proteose Peptone for growth promotion and toxin production with a wide range of fastidious microorganisms.

User Quality Control

Identity Specifications

Bacto™ Proteose Peptone

Dehydrated Appearance:	Tan, free-flowing, granules.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is light amber, clear to very slightly opalescent, may have a slight precipitate. 2.0% solution is light to medium amber, clear to slightly opalescent, may have a slight precipitate. 10.0% solution is medium dark amber, slightly opalescent to opalescent, may have a slight precipitate.
Reaction of 1.0% Solution at 25°C:	pH 6.6-7.6

BiTek™ Proteose Peptone

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light amber, clear to very slightly opalescent, may have a slight precipitate. 2.0% solution is light amber, clear to slightly opalescent, may have a slight precipitate. 10.0% solution is light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 1.0% Solution at 25°C:	pH 6.5-7.5

Bacto™ Proteose Peptone No. 2

Dehydrated Appearance:	Tan, free-flowing, granules.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is light to medium amber, clear. 2.0% solution is medium amber, clear. 10.0% solution is medium to dark amber, slightly opalescent to opalescent, may have a slight precipitate.
Reaction of 1.0% Solution at 25°C:	pH 7.2-7.6

Bacto™ Proteose Peptone No. 3

Dehydrated Appearance:	Golden tan, free-flowing granules.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light amber, clear to very slightly opalescent, may have a slight precipitate. 2.0% solution is light amber, clear to very slightly opalescent, may have a slight precipitate. 10.0% solution is light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 1.0% Solution at 25°C:	pH 7.0-7.6

Bacto™ Proteose Peptone No. 4

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light amber, clear to very slightly opalescent, may have a slight precipitate. 2.0% solution is light amber, clear to slightly opalescent, may have a slight precipitate. 10.0% solution is medium amber, slightly opalescent to opalescent, may have a slight precipitate.
Reaction of 1.0% Solution at 25°C:	pH 6.6-7.6

Continued

Principles of the Procedure

Bacto Proteose Peptone, **BiTek** Proteose Peptone, **Bacto** Proteose Peptone No. 2, **Bacto** Proteose Peptone No. 3 and **Bacto** Proteose Peptone No. 4 are enzymatic digests of protein that provide nitrogen in a form that is readily available for bacterial growth.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** Proteose Peptone, **BiTek** Proteose Peptone, **Bacto** Proteose Peptone No. 2, **Bacto** Proteose Peptone No. 3 and **Bacto** Proteose Peptone No. 4 in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for procedures using **Bacto** Proteose Peptone, **BiTek** Proteose Peptone, **Bacto** Proteose Peptone No. 2, **Bacto** Proteose Peptone No. 3 and **Bacto** Proteose Peptone No. 4.

Expected Results

Refer to appropriate references and procedures for results.

Cultural Response

Biochemical Reactions

Bacto™ Proteose Peptone, BiTek™ Proteose Peptone, Bacto™ Proteose Peptone No. 2, Bacto™ Proteose Peptone No. 3 or Bacto™ Proteose Peptone No. 4

Prepare a sterile solution as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922	~10^7	Negative
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	0.1 mL, undiluted	Positive

Growth Response

Bacto™ Proteose Peptone, BiTek™ Proteose Peptone or Bacto™ Proteose Peptone No. 4

1. Prepare a sterile solution with 2% **Bacto** Proteose Peptone, **BiTek** Proteose Peptone or **Bacto** Proteose Peptone No. 4, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	30-300	Good*
Staphylococcus aureus	25923	30-300	Good
Streptococcus pneumoniae	6303	30-300	Good

Fair to good for **BiTek Proteose Peptone.*

2. For **Bacto** Proteose Peptone and **Bacto** Proteose Peptone No. 4 only, prepare KL Virulence Agar from individual ingredients using 2 g of **Bacto** Proteose Peptone or **Bacto** Proteose Peptone No. 4. Sterilize, cool to 55-60°C and add KL Virulence Enrichment and Tellurite Solution. Dispense into Petri dishes. Inoculate with a loopful of surface growth and incubate at 35 ± 2°C for 72 hours. Examine at 24, 48 and 72 hours for growth and blackening.

ORGANISM	ATCC™	RESULT
Corynebacterium diphtheriae biotype intermedius	8032	Growth
Corynebacterium diphtheriae biotype gravis	8028	Growth
Corynebacterium diphtheriae biotype mitis	8024	Growth

Bacto™ Proteose Peptone No. 2

Prepare a sterile solution with 2% **Bacto** Proteose Peptone No. 2, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	30-300	Good
Staphylococcus aureus	25923	30-300	Good

Bacto™ Proteose Peptone No. 3

Prepare a sterile solution with 2% **Bacto** Proteose Peptone No. 3, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Staphylococcus aureus	25923	30-300	Good
Streptococcus pneumoniae	6303	30-300	Good
Streptococcus pyogenes	19615	30-300	Good

References

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3. Nelson. 1927. J. Infect. Dis. *41*:9.
4. Mollby and Holme. 1976 J. Gen. Microbiol. *96*:137.
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11. Jan, Jones, Emery and Al-Rubeai. 1994. Cytotechnol. *16*:17.
12. Shukla, Kaul and Mehlotra. 1989. Indian J. Exp. Biol. *27*:785.
13. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
14. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.
15. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, USEPA, Washington, D.C.
16. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
17. Bunney and Thomas. 1936. J. Immunol. *31*:95.
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19. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Bacto™ Proteose Peptone

EPA SMD SMWW USDA

Cat. No. 211684 Dehydrated – 500 g
212010 Dehydrated – 10 kg

BiTek™ Proteose Peptone

Cat. No. 253310 Dehydrated – 10 kg

Bacto™ Proteose Peptone No. 2

Cat. No. 212120 Dehydrated – 500 g
212110 Dehydrated – 10 kg

Bacto™ Proteose Peptone No. 3

BAM EPA SMWW USDA

Cat. No. 211693 Dehydrated – 500 g
212220 Dehydrated – 2 kg
212230 Dehydrated – 10 kg

Bacto™ Proteose Peptone No. 4

Cat. No. 211715 Dehydrated – 10 kg

Proteose No. 3 Agar

Intended Use
Proteose No. 3 Agar is used with added enrichment in isolating and cultivating *Neisseria* and *Haemophilus*.

Summary and Explanation
Proteose No. 3 Agar, introduced in 1938, is used for isolating *Neisseria gonorrhoeae*. When enriched with Hemoglobin and Supplement B,[1,2] Proteose No. 3 Agar recovers gonococci in a manner comparable to more complex media, ranking only slightly lower than GC media at 24 hours.[3]

Chocolate agar may be prepared from Proteose No. 3 Agar with the addition of 2% Hemoglobin. Hemoglobin provides X factor (hemin), required for growth of *Haemophilus* and enhanced growth of *Neisseria*.

The growth rate of *Neisseria* and *Haemophilus* spp. may be improved with the addition of 1% Supplement B or VX, which provide the growth factors glutamine and cocarboxylase.

Principles of the Procedure
Proteose Peptone No. 3 provides nitrogen, vitamins and amino acids. Dextrose is a carbon source. Sodium chloride maintains the osmotic balance in the medium, which is buffered by disodium phosphate. Agar is the solidifying agent.

Proteose Peptone No. 3 Agar is intended for use with supplementation by 2% Hemoglobin and Supplement B or Supplement VX.

Formula
Difco™ Proteose No. 3 Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	20.0	g
Dextrose	0.5	g
Sodium Chloride	5.0	g
Disodium Phosphate	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 45.5 g of the powder in 500 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50-60°C.
4. Aseptically add 500 mL sterile 2% solution of Hemoglobin and 10 mL of Supplement B or VX. Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
For a complete discussion of the isolation and identification of *Haemophilus* or *Neisseria* spp., refer to the procedures outlined in the references.[4-6]

User Quality Control
Identity Specifications
Difco™ Proteose No. 3 Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	9.1% (double strength) solution, soluble in purified water upon boiling with frequent agitation. Solution is light to medium amber, opalescent with a slight flocculent precipitate.
Prepared Appearance:	(Single strength) light amber, opalescent with a slight flocculent precipitate.
Reaction of 9.1% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response
Difco™ Proteose No. 3 Agar

Prepare the medium per label directions (with 2% Hemoglobin and 1% Supplement B). Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Haemophilus influenzae	10211	10^2-10^3	Good
Neisseria gonorrhoeae	43070	10^2-10^3	Good
Neisseria meningitidis	13102	10^2-10^3	Good
Neisseria sicca	9913	10^2-10^3	Good

Expected Results
Refer to appropriate references and procedures for results.

Limitation of the Procedure
Proteose No. 3 Agar is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.

References

1. Lankford, Scott, Cox and Cooke. 1943. J. Bacteriol. *45*:321.
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4. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
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Availability

Difco™ Proteose No. 3 Agar

Cat. No. 265100 Dehydrated – 500 g

BBL™ Hemoglobin Solution 2%

Cat. No. 211874 Bottle – 10 × 100 mL*

BBL™ Hemoglobin, Bovine, Freeze-Dried

Cat. No. 212392 Dehydrated – 500 g

Difco™ Supplement B

Cat. No. 227610 Lyophilized – 6 × 10 mL with Reconstituting Fluid*
227620 Lyophilized – 100 mL with Reconstituting Fluid*

Difco™ Supplement VX

Cat. No. 233541 Lyophilized – 6 × 10 mL with Reconstituting Fluid*
233542 Lyophilized – 100 mL with Reconstituting Fluid*

**Store at 2-8°C.*

Pseudomonas Agars
Pseudomonas Agar F • Flo Agar
Pseudomonas Agar P • Tech Agar

Intended Use

Pseudomonas Agar F, also known as Flo Agar, is used for the enhancement of fluorescin production and Pseudomonas Agar P, also known as Tech Agar, is used for the enhancement of pyocyanin production by *Pseudomonas*.

Summary and Explanation

Pseudomonas aeruginosa is widely distributed in soil, water and foods. It is frequently isolated from infusion fluids, disinfectants and cosmetics. The organism causes disease in humans; e.g., ocular infections, burn wound infections and respiratory tract infections.[1]

Most strains of *P. aeruginosa* produce pyocyanin, a blue, water- and chloroform-soluble, nonfluorescent pigment that diffuses into the surrounding medium.[2] *P. aeruginosa* is the only *Pseudomonas* species known to produce this pigment. (However, certain strains are apyocyanogenic.)

Some strains of *P. aeruginosa* produce other pigments, such as the brown-black pyomelanin, the red pyorubin or the yellow pyoverdin. Pyoverdin is a water soluble fluorescent pigment often produced by *P. aeruginosa* and other pseudomonads isolated from humans.[2] The presence of these pigments can, however, mask the production of pyocyanin.[2]

Pseudomonas Agar F (Flo Agar) and Pseudomonas Agar P (Tech Agar) are modifications of two media (Medium A and Medium B) that King et al. developed to enhance pigment production for improved differentiation of pseudomonads.[3]

Principles of the Procedure

The 1:1 ratio of casein to meat peptone in Pseudomonas Agar F (Flo Agar) is conducive to fluorescin production by *Pseudomonas*. These peptones contain phosphorus, which is stimulatory to fluorescin production.[4] The addition of dipotassium phosphate increases the phosphorus content of the medium, thereby enhancing production of the fluorescent pigment. Magnesium sulfate provides essential ions for fluorescin production.[4]

Pseudomonas Agar P (Tech Agar) contains enzymatic digest of gelatin to provide amino acids and other essential nitrogenous substances. The gelatin peptone is low in phosphorous to minimize the inhibitory action on pyocyanin production.[4] Magnesium, potassium and sulfate ions promote pyocyanin production.[4]

Both media contain glycerol, which acts as a source of energy and enhances pigment production.

Formulae

Difco™ Pseudomonas Agar F

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Proteose Peptone No. 3	10.0	g
Dipotassium Phosphate	1.5	g
Magnesium Sulfate	1.5	g
Agar	15.0	g

Difco™ Pseudomonas Agar P

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	20.0	g
Magnesium Chloride	1.4	g
Potassium Sulfate	10.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water containing 10 g of glycerol:
   Difco™ Pseudomonas Agar F – 38 g;
   Difco™ Pseudomonas Agar P – 46.4 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Specimens must first be isolated in pure culture on an appropriate medium. The isolate should be Gram-stained and examined to confirm that morphology is appropriate for *Pseudomonas*.

User Quality Control

Identity Specifications

Difco™ Pseudomonas Agar F

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water with 1% glycerol upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 3.8% Solution with 1% glycerol at 25°C:	pH 7.0 ± 0.2

Difco™ Pseudomonas Agar P

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.64% solution, soluble in purified water with 1% glycerol upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.64% Solution with 1% glycerol at 25°C:	pH 7.0 ± 0.2

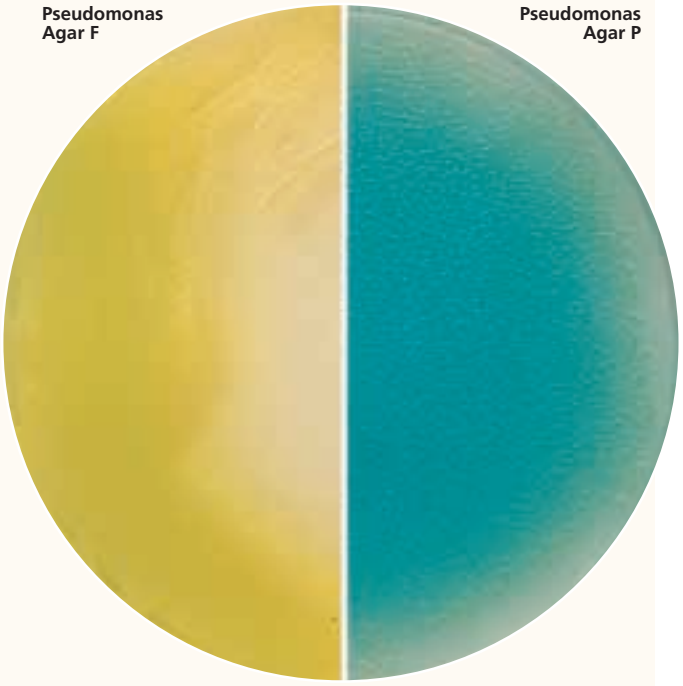
Cultural Response

Difco™ Pseudomonas Agar F or Pseudomonas Agar P

Prepare the medium per label directions. For Pseudomonas Agar F inoculate as described below and incubate at 35 ± 2°C for 18-24 hours. For Pseudomonas Agar P inoculate with fresh cultures and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU PSEUDOMONAS AGAR F	RECOVERY	PIGMENT PRODUCTION PSEUDOMONAS AGAR F	PIGMENT PRODUCTION PSEUDOMONAS AGAR P
Pseudomonas aeruginosa	9027	30-300	Good	Greenish yellow	Blue
Pseudomonas aeruginosa	27853	30-300	Good	Greenish yellow	Blue to green
Pseudomonas cepacia	25609	30-300	Good	No pigment	No pigment

Pseudomonas aeruginosa
ATCC™ 9027



OP

Using a sterile inoculating loop or needle, streak plates or slants with several colonies from the subculture medium. Incubate plates or tubes, with caps loosened, at 35 ± 2°C for 18-24 hours. If the isolate fails to grow or grows slowly, reincubate at 25-30°C for 1-2 days and observe for growth and pigment production.[5]

Expected Results

Examine Pseudomonas Agar F (Flo Agar) under long wavelength UV light (366 nm) for fluorescin, a greenish-yellow fluorescent pigment in the colonies and surrounding medium. Examine Pseudomonas Agar P (Tech Agar) for pyocyanin, a blue to blue-green pigment seen in the colonies and surrounding medium. Confirm the presence of pyocyanin by adding several drops of chloroform and observe for a blue color in the chloroform. (Pyocyanin is more soluble in chloroform than in water.)

Limitations of the Procedure

1. Occasionally, a *Pseudomonas* culture is encountered that will produce small amounts of pigment in the medium. When this happens, a yellow-green color will appear on Pseudomonas Agar F (Flo Agar) or a blue-green color on Pseudomonas Agar P (Tech Agar). If a blue-green color occurs on Pseudomonas Agar P (Tech Agar), confirmation of the presence of pyocyanin can be made by extraction with chloroform ($CHCl_3$).[4]
2. The formation of nonpigmented colonies does not completely rule-out *a Pseudomonas aeruginosa* isolate.
3. A pyocyanin-producing *Pseudomonas* strain will usually also produce fluorescin. It must, therefore, be differentiated from other simple fluorescent pseudomonads by other means. Temperature can be a determining factor as most other fluorescent strains will not grow at 35°C. Rather, they grow at 25-30°C.[4]

References

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4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Sewell. 1987. *In* Wentworth (ed.), Diagnostic procedures for bacterial infections, 7th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Pseudomonas Agar F

BAM CCAM

Cat. No. 244820 Dehydrated – 500 g

BBL™ Flo Agar

BAM CCAM

Cat. No. 296003 Prepared Slants – Pkg. of 10

Difco™ Pseudomonas Agar P

BAM

Cat. No. 244910 Dehydrated – 500 g

Europe
Cat. No. 257018 Prepared Plates – Pkg. of 20*

BBL™ Tech Agar

BAM

Cat. No. 296004 Prepared Slants – Pkg. of 10

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

**Store at 2-8°C.*

Pseudomonas Isolation Agar

Intended Use

Pseudomonas Isolation Agar is used with added glycerol in isolating *Pseudomonas* and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on pigment formation.

Summary and Explanation

Pseudomonas aeruginosa is an opportunistic pathogen that can infect eyes, ears, burns and wounds.[1] It is also a leading cause of hospital acquired infections. Patients undergoing antibiotic therapy are especially susceptible to infection by *Pseudomonas aeruginosa*.

Pseudomonas Isolation Agar is prepared according to a slight modification of the Medium A formulation of King, Ward and Raney.[2] Pseudomonas Isolation Agar includes Irgasan™, a potent broad spectrum antimicrobial that is not active against *Pseudomonas*.[3] As well as being selective, Pseudomonas Isolation Agar is formulated to enhance the formation of the blue or blue-green pyocyanin pigment by *Pseudomonas aeruginosa*. The pigment diffuses into the medium surrounding growth.

Irgasan™ is a trademark of Ciba-Geigy.

Principles of the Procedure

Peptone provides the carbon and nitrogen necessary for bacterial growth. Magnesium chloride and potassium sulfate promote production of pyocyanin. Irgasan, an antimicrobial agent, selectively inhibits gram-positive and gram-negative bacteria other than *Pseudomonas* spp. Agar is the solidifying agent. Glycerol serves as an energy source and also helps to promote pyocyanin production.

Formula

Difco™ Pseudomonas Isolation Agar

Approximate Formula* Per Liter

Peptone	20.0	g
Magnesium Chloride	1.4	g
Potassium Sulfate	10.0	g
Irgasan™	25.0	mg
Agar	13.6	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water containing 20 mL of glycerol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Pseudomonas Isolation Agar

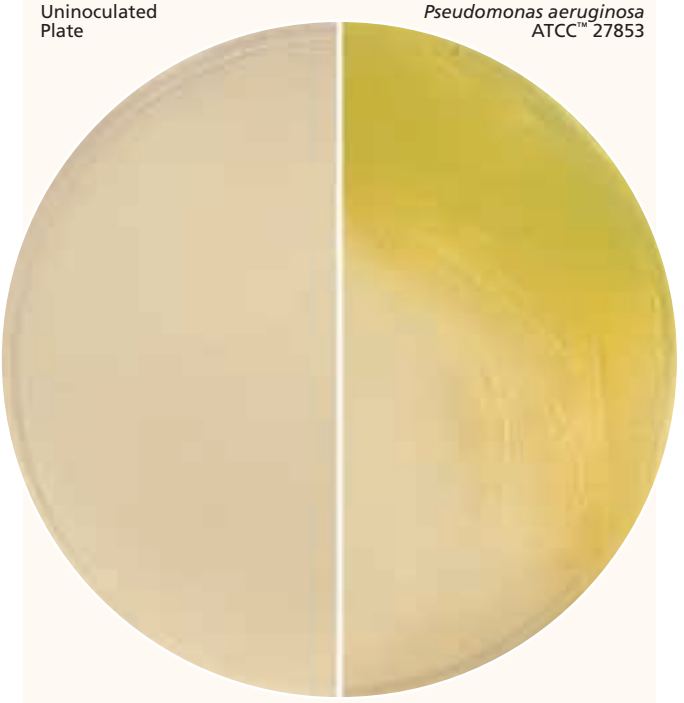
Dehydrated Appearance:	Very light beige, homogeneous, free-flowing.
Solution:	4.5% solution, soluble in purified water containing 2% glycerol upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 4.5% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Pseudomonas Isolation Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition	–
Pseudomonas aeruginosa	10145	10^2-10^3	Good	Green to blue-green
Pseudomonas aeruginosa	27853	10^2-10^3	Good	Green to blue-green



Procedure
Inoculate the medium using the streak plate method to obtain isolated colonies. Incubate for 18-48 hours at 35 ± 2°C.

Expected Results
Examine for the presence of good growth. *Pseudomonas aeruginosa* colonies may be greenish after incubation for 18 hours and turn blue to blue-green as incubation continues up to 24-48 hours, with diffusion of the pigment into the medium.

Limitations of the Procedure
1. Some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.[1,4]
2. Non-*Pseudomonas aeruginosa* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Pseudomonas aeruginosa*. Consult appropriate references.[1,5]

References

1. Kiska and Gilligan. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. King, Ward and Raney. 1954. J. Lab. Clin. Med. *44*:301.
3. Furia and Schenkel. January, 1968. Soap and Chemical Specialties.
4. Gaby and Free. 1931. J. Bacteriol. *22*:349.
5. Isenberg and Garcia (ed.). 2004 (update, 2007) Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability
Difco™ Pseudomonas Isolation Agar

Cat. No. 292710 Dehydrated – 500 g

Europe

Cat. No. 257002 Prepared Plates – Pkg. of 20*

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

**Store at 2-8°C.*

OP

Pseudosel™ Agar

(See Cetrimide Agar Base)

Purple Agar Base • Purple Broth Base Purple Broth with Carbohydrates

Intended Use
Purple Agar Base and Purple Broth Base are used with added carbohydrate in differentiating pure cultures of bacteria. They are used primarily for the differentiation and presumptive identification of gram-negative enteric bacilli based on patterns of carbohydrate fermentation.

Summary and Explanation
Purple Agar Base and Purple Broth Base are carbohydrate-free media with a slightly acid pH that, when supplemented with carbohydrates, are useful in obtaining accurate fermentation reactions, particularly in the identification of gram-negative enteric bacteria.[1,2] The media either may be used with the addition of the appropriate carbohydrate or the plain broth may be used with **BBL™ Taxo™** Carbohydrate Discs.

Principles of the Procedure
These media consist of carbohydrate-free peptone with the pH indicator bromcresol purple. Specific carbohydrates are added in a concentration of 0.5-1%. This concentration is recommended to ensure against depletion of the carbohydrate and reversal of the fermentation reaction.

When the media are inoculated with an organism that is able to ferment the carbohydrate present, acid or acid and gas are produced. A Durham tube is provided in tubed broth media to collect the gas produced during fermentation. The indicator in the media changes from purple to yellow when the amount of acid produced by carbohydrate fermentation is greater than the alkaline end products from peptone utilization. If the carbohydrate is not fermented, the color will remain unchanged or become more alkaline (darker purple) due to degradation of the amino acids in the medium.

Formulae
Difco™ Purple Agar Base

Approximate Formula* Per Liter

Proteose Peptone No. 3	10.0	g
Beef Extract	1.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Bromcresol Purple	0.02	g

BBL™ Purple Broth Base

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	10.0	g
Sodium Chloride	5.0	g
Bromcresol Purple	0.02	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
Difco™ Purple Agar Base

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
   NOTE: When preparing 0.5-1% carbohydrate fermentation agars, dissolve 5-10 g of the desired carbohydrate in the basal

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Purple Agar Base

Dehydrated Appearance:	Light tan with grayish-green cast, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water upon boiling. Solution is purple, slightly opalescent.
Prepared Appearance:	Purple, slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Purple Agar Base

Prepare the medium per label directions with 1% dextrose. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	ACID	GAS
Alcaligenes faecalis	8750	Good	–	–
Escherichia coli	25922	Good	+	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	+	+

+ = yellow for acid; - = no change

Identity Specifications

BBL™ Purple Broth Base

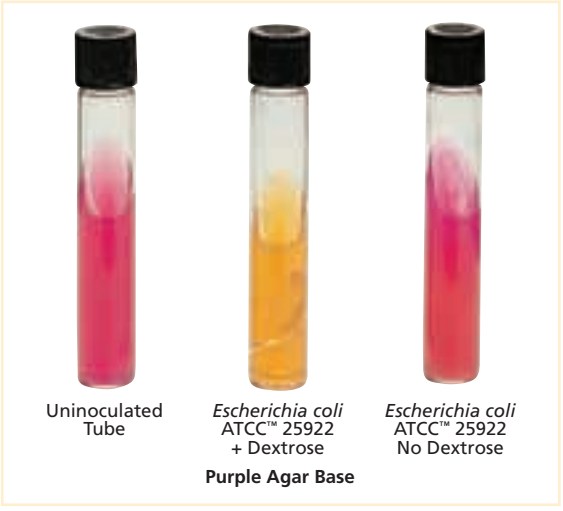
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.5% solution, soluble in purified water. Solution is medium purple, clear to slightly hazy.
Prepared Appearance:	Medium purple, clear to slightly hazy.
Reaction of 1.5% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

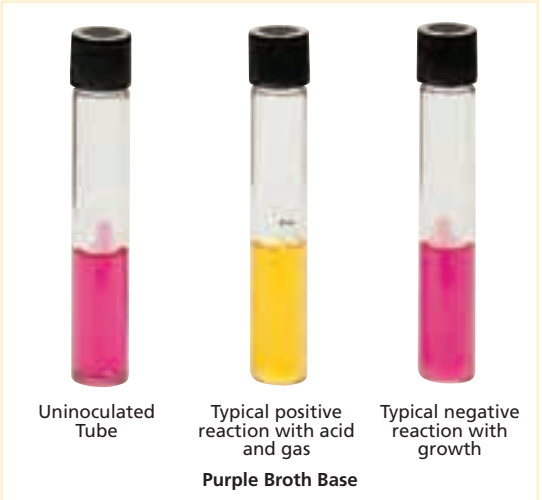
BBL™ Purple Broth Base

Prepare the medium per label directions. Inoculate with fresh cultures diluted 1:10 and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	RECOVERY	REACTION
Escherichia coli	25922	Good	Alkaline (purple)
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	Alkaline (purple)



Purple Agar Base



Purple Broth Base

medium prior to autoclaving, or dissolve 31 g of Purple Agar Base in 900 mL of purified water and aseptically add 100 mL of a sterile 5-10% carbohydrate solution (w/v) after autoclaving and cooling the basal medium.

4. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Purple Broth Base

1. Suspend 15 g of the powder in 1 L of purified water. Add carbohydrates, 5-10 g/L, if desired, and readjust the pH if necessary.
2. Dispense with Durham fermentation tubes, if gas formation is to be recorded.
3. Autoclave at 118°C for 15 minutes. DO NOT OVERHEAT.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Carbohydrates should be added to the basal medium either as sterile solutions or as **BBL Taxo** Carbohydrate Discs (broth).

Inoculate tubes of carbohydrate agar with an inoculating needle to within 1/4 inch from the bottom of the tube. Inoculate tubes of broth containing an appropriate carbohydrate using a light inoculum from an 18- to 24-hour pure culture. Incubate tubes for 24-72 hours or up to 30 days at 35 ± 2°C either in an aerobic or anaerobic atmosphere depending on the organism being tested.

Expected Results

Examine the tubes daily for growth. A yellow color (acid) is a positive reaction for fermentation of the carbohydrate incor-

porated into the medium. Bubbles in the inverted fermentation vials are an indication of gas production.

Consult appropriate texts for expected reactions of specific organisms with specific carbohydrates.[1-4]

References

1. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co, Inc., New York, N.Y.
2. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Purple Agar Base

Cat. No. 222810 Dehydrated – 500 g

BBL™ Purple Broth Base

AOAC BAM CCAM COMPF ISO SMD USDA

Cat. No. 211558 Dehydrated – 500 g
296012 Prepared Tubes (K Tubes) with Durham Tube – Pkg. of 10*

BBL™ Purple Broth with Carbohydrates and Durham Tube

Cat. No. 295863 Prepared Tubes (K Tubes) with Arabinose – Pkg. of 10*
295864 Prepared Tubes (K Tubes) with Cellobiose – Pkg. of 10*
296013 Prepared Tubes (K Tubes) with Dextrose – Pkg. of 10*
295865 Prepared Tubes (K Tubes) with Dulcitol – Pkg. of 10*
297734 Prepared Tubes (K Tubes) with Fructose – Pkg. of 10*
296014 Prepared Tubes (K Tubes) with Galactose –Pkg. of 10*
295866 Prepared Tubes (K Tubes) with Inositol – Pkg. of 10*
296015 Prepared Tubes (K Tubes) with Lactose – Pkg. of 10*
295999 Prepared Tubes (K Tubes) with Maltose – Pkg. of 10*
297018 Prepared Tubes (K Tubes) with Mannitol – Pkg. of 10*
295867 Prepared Tubes (K Tubes) with Raffinose – Pkg. of 10*
297203 Prepared Tubes (K Tubes) with Rhamnose – Pkg. of 10*
297019 Prepared Tubes (K Tubes) with Salicin – Pkg. of 10*
297020 Prepared Tubes (K Tubes) with Sorbitol – Pkg. of 10*
296016 Prepared Tubes (K Tubes) with Sucrose – Pkg. of 10*
295870 Prepared Tubes (K Tubes) with Trehalose – Pkg. of 10*
295871 Prepared Tubes (K Tubes) with Xylose – Pkg. of 10*

*Store at 2-8°C.

OP

Pyridoxine Y Medium

Intended Use

Pyridoxine Y Medium is used for determining pyridoxine concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimum growth of the test organism except the single essential vitamin to be determined.

Pyridoxine Y Medium is patterned after the formulation of Campling and Nixon,[1] and modified by Hurley[2] and Parrish, Loy and Kline.[3] This medium is used in the microbiological assay of pyridoxine using *Saccharomyces cerevisiae* ATCC™ 9080 as the test organism.

Principles of the Procedure

Pyridoxine Y Medium is free from pyridoxine, but contains all other nutrients and vitamins essential for the growth of *S. cerevisiae* ATCC 9080. The addition of pyridoxine in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Pyridoxine Y Medium

Approximate Formula* Per Liter		
Dextrose	40.0	g
L-Asparagine	4.0	g
Ammonium Sulfate	4.0	g
Monopotassium Phosphate	3.0	g
Magnesium Sulfate	1.0	g
Calcium Chloride	0.49	g
DL-Methionine	40.0	mg
DL-Tryptophan	40.0	mg
DL-Isoleucine	40.0	mg
DL-Valine	40.0	mg
L-Histidine Hydrochloride	20.0	mg
Riboflavin	20.0	mg
Biotin Salt	8.0	mg
Inositol	5.0	mg
Ferrous Sulfate	500.0	µg
Thiamine Hydrochloride	400.0	µg
Calcium Pantothenate	400.0	µg
Nicotinic Acid	400.0	µg
Boric Acid	200.0	µg
Potassium Iodide	200.0	µg
Ammonium Molybdate	40.0	µg
Manganese Sulfate	80.0	µg
Copper Sulfate	90.0	µg
Zinc Sulfate	80.0	µg

*Adjusted and/or supplemented as required to meet performance criteria.

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilizing and cooling conditions uniform throughout assay.

User Quality Control

Identity Specifications

Difco™ Pyridoxine Y Medium

Dehydrated Appearance:	White to off-white, fine, free-flowing, homogeneous.
Solution:	5.3% (double strength) solution, soluble in purified water upon boiling for 2-3 minutes. Solution 2.65% (single strength) is colorless to very, very light amber, clear, may have a slight precipitate.
Prepared Appearance:	(Single strength) colorless to very light amber, clear, may have a slight precipitate.
Reaction of 2.65% Solution at 25°C:	pH 4.4 ± 0.2

Cultural Response

Difco™ Pyridoxine Y Medium

Prepare the medium per label directions. The medium supports the growth of *Saccharomyces cerevisiae* ATCC™ 9080 when prepared in single strength and supplemented with pyridoxine hydrochloride. The medium should produce a standard curve when tested using a pyridoxine hydrochloride reference standard at 0.0 to 10.0 ng per 10 mL. Incubate the tubes with caps loosened at 25-30°C for 22 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Directions for Preparation from Dehydrated Product

1. Suspend 5.3 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense in 5 mL amounts into flasks, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust flask volume to 10 mL with purified water.
6. Steam at 100°C for 10 minutes.

Procedure

Stock cultures of *S. cerevisiae* ATCC 9080 are carried on Lactobacilli Agar AOAC. Following incubation at 25-30°C (held constant within ± 0.5°C) for 18-24 hours, store the cultures in the dark at 2-8°C. Prepare fresh slant cultures every week. Do not use stock cultures for preparing the inoculum if more than one week old. Inoculum for assay is prepared by subculturing a stock culture of *S. cerevisiae* ATCC 9080 into a tube (10 mL) of single strength Pyridoxine Y Medium containing 1 ng per mL each of pyridoxal hydrochloride, pyridoxamine dihydrochloride and pyridoxine hydrochloride. After 18-24 hours incubation at 25-30°C (held constant within ± 0.5°C), centrifuge the cells under aseptic conditions and decant the liquid supernatant. Wash the cells 3× with 10 mL sterile 0.85% saline. After the third wash, resuspend in 10 mL sterile single strength medium and adjust to a turbidity of 45-50% transmittance when read on the spectrophotometer at 660 nm.

It is essential that a standard curve be set up for each separate assay. Conditions of steaming and temperature of incubation which influence the standard curve readings cannot always be duplicated. Obtain the standard curve by using pyridoxine hydrochloride at levels of 0, 1, 2, 4, 6, 8 and 10 ng per flask (10 mL).

The concentrations of pyridoxine hydrochloride required for the preparation of the standard curve may be prepared as follows:

1. Dissolve 50 mg dried pyridoxine hydrochloride in about 100 mL of 1 N HCl.
2. Dilute to 500 mL with additional 1 N HCl.
3. Further dilute by adding 2 mL to 998 mL purified water to make a stock solution containing 200 ng pyridoxine hydrochloride per mL. Prepare the stock solution fresh daily.

To make the standard solution, dilute 1 mL of stock solution with 99 mL purified water, to make a solution containing 2 ng pyridoxine hydrochloride per mL. Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL per assay tube.

Following inoculation, incubate the tubes on a shaker (about 100 rpm) at 25-30°C for 22 hours. Steam in the autoclave for 5 minutes at 100°C to stop growth. Measure the growth turbidimetrically using a spectrophotometer at any specific wavelength between 540 and 660 nm.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ± 10% from the average and use the results only if two-thirds of the values do not vary more than ± 10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be grown and maintained on a medium recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. Campling and Nixon. 1954. J. Physiol. *126*:71.
2. Hurley. 1960. J. Assoc. Off. Agri. Chem. *43*:43.
3. Parrish, Loy and Kline. 1956. J. Assoc. Off. Agri. Chem. *39*:157.

Availability

Difco™ Pyridoxine Y Medium

Cat. No. 295110 Dehydrated – 100 g*

**Store at 2-8°C.*

R2A Agar

Intended Use

R2A Agar is used for enumerating heterotrophic organisms in treated potable water.

Summary and Explanation

R2A Agar was developed by Reasoner and Geldreich[1] for bacteriological plate counts of treated potable water. A low nutrient medium, such as R2A Agar, in combination with a lower incubation temperature and longer incubation time stimulates the growth of stressed and chlorine-tolerant bacteria.[1] Nutritionally rich media, such as Plate Count Agar (Standard Methods Agar), support the growth of fast-growing bacteria but may suppress slow growing or stressed bacteria found in treated water. When compared with nutritionally rich media, R2A Agar has been reported to improve the recovery of stressed and chlorine-tolerant bacteria from drinking water systems.[2-4]

R2A Agar is recommended in standard methods for pour plate, spread plate and membrane filter methods for heterotrophic plate counts.[5,6]

Principles of the Procedure

Yeast extract provides a source of trace elements and vitamins. Peptone and casamino acids provide nitrogen, vitamins, amino acids, carbon and minerals. Dextrose serves as a carbon source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Sodium pyruvate increases the recovery of stressed cells. Potassium phosphate is used to balance the pH and provide phosphate. Magnesium sulfate is a source of divalent cations and sulfate. Agar is the solidifying agent.

User Quality Control

Identity Specifications

Difco™ R2A Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.82% solution, soluble in purified water upon boiling. Solution is light amber, very slightly opalescent with a slight precipitate.
Prepared Appearance:	Light amber, slightly opalescent with a slight precipitate.
Reaction of 1.82% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ R2A Agar

Prepare the medium per label directions. Inoculate samples using the spread plate method. Incubate at 35 ± 2°C for 42-48 hours. Recovery should be typical compared to a previously approved (control) lot and greater than parallel plates of Plate Count Agar (Standard Methods Agar).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	30-300	Good
Escherichia coli	25922	30-300	Good
Pseudomonas aerginosa	27853	30-300	Good
Staphylococcus aureus	25923	30-300	Good

Formula

Difco™ R2A Agar

Approximate Formula* Per Liter		
Yeast Extract	0.5	g
Proteose Peptone No. 3	0.5	g
Casamino Acids	0.5	g
Dextrose	0.5	g
Soluble Starch	0.5	g
Sodium Pyruvate	0.3	g
Dipotassium Phosphate	0.3	g
Magnesium Sulfate	0.05	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 18.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Water samples should be collected as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9060A.[5] To minimize changes in bacterial population, water samples should be tested as soon as possible, but at least within six hours of collection if the sample has not been refrigerated or within 30 hours if refrigerated.
2. Prepare test dilutions for heterotrophic plate count.
3. Plate the test sample and dilutions by the spread plate, pour plate or membrane filter method. Do not exceed 1 mL of sample or dilution per spread or pour plate. The volume of test sample to be filtered for the membrane filter technique will vary.
4. Maintain proper humidity during prolonged incubation:

INCUBATION TEMPERATURE	MINIMUM INCUBATION TIME[3]	OPTIMAL INCUBATION TIME[3]
35°C	72 hours	5-7 days
20 or 28°C	5 days	7 days

Expected Results

Count colonies on spread or pour plates demonstrating 30-300 colonies per plate or 20-200 colonies when using the membrane filter method. Compute bacterial count per mL of sample by multiplying the average number of colonies per plate by the reciprocal of the appropriate dilution.

Report counts as colony forming units (CFU) per mL and report variables of incubation such as temperature and length of time.

Limitations of the Procedure

1. R2A Agar is intended for use only with treated potable water since it is recommended for compromised bacteria.
2. Use of the pour plate method is discouraged because recovery of stressed bacteria may be compromised by the heat shock (44-46°C) and low oxygen tension that are part of the procedure.[7,8]
3. Incubation time longer than indicated may be necessary to recover additional slow-growing bacteria.
4. R2A Agar performs best with the spread plate technique; however, that procedure is limited to a small sample volume.
5. Fast-growing bacteria may produce smaller size colonies on R2A Agar than on nutritionally rich media.
6. R2A Agar is a low nutrient medium intended for culturing compromised microorganisms. Good growth of standard, healthy control organisms does not necessarily reflect the ability of the medium to recover stressed organisms. Each new lot of medium should be performance tested against a previous lot of R2A Agar using tap water.

References

1. Reasoner and Geldreich. 1985. Appl. Environ. Microbiol. *49*:1.
2. Fiksdal, Vik, Mills and Staley. 1982. J. Am. Water Works Assoc. *74*:313.
3. Kelly, Justice and Nagy. 1983. Abstr. Q122, p. 280. Abstr. 83rd Annu. Meet. Am. Soc. Microbiol. 1983.
4. Means, Hanami, Ridgway and Olson. 1981. J. Am. Water Works Assoc. *53*:585.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
6. Kim and Feng. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Van Soestberger and Lee. 1969. Appl. Microbiol. *18*:1092.
8. Klein and Wu. 1974. Appl. Microbiol. *27*:429.

Availability

Difco™ R2A Agar

COMPF EPA SMWW

Cat. No.	218262	Dehydrated – 100 g
	218263	Dehydrated – 500 g
	218261	Dehydrated – 2 kg

BBL™ R2A Agar

COMPF EPA SMWW

Cat. No.	299436	Prepared Bottles, 500 mL – Pkg. of 10
Europe		
Cat. No.	257008	Prepared Plates – Pkg. of 20*
	257073	Prepared Plates – Ctn. of 120*
	257336	Prepared Bottles, 100 mL – Pkg. of 25
Japan		
Cat. No.	251258	Prepared Plates – Pkg. of 20*
	251259	Prepared Plates – Ctn. of 100*
Mexico		
Cat. No.	252737	Prepared Plates – Pkg. of 10*
	252738	Prepared Plates (60 mm) – Pkg. of 20*

**Store at 2-8°C.*

Raka-Ray No. 3 Medium

Intended Use

Raka-Ray No. 3 Medium is recommended for the isolation of lactic acid bacteria encountered in beer and the brewing process.

User Quality Control

Identity Specifications

Difco™ Raka-Ray No. 3 Medium

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	7.49% solution, soluble in purified water with 1% polysorbate 80 upon boiling. Solution is medium to dark amber, clear to very slightly opalescent.
Prepared Appearance:	Medium to dark amber, clear to slightly opalescent.
Reaction of 7.49% Solution at 25°C:	pH 5.4 ± 0.2

Cultural Response

Difco™ Raka-Ray No. 3 Medium

Prepare the medium per label directions (with the addition of 3 g/L phenylethanol and 7 mg/L cycloheximide, adjusted for potency). Inoculate, overlay with 4 mL of sterile medium and incubate anaerobically at 27-30°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-2×10^3	None to poor
Lactobacillus brevis	367	30-300	Good
Lactobacillus buchneri	11307	30-300	Good
Pediococcus acidilactici	8042	30-300	Good

Summary and Explanation

Spoilage organisms are often seriously detrimental to beer flavor. Lactic acid bacteria including lactobacilli and pediococci, which can cause spoilage, are physiologically very diverse.

Raka-Ray No. 3 Medium was developed from a formulation suggested by Saha, Sondag and Middlekauff,[1] who tested a range of ingredients for their ability to stimulate growth of lactic acid bacteria. Polysorbate 80, liver extract, maltose, N-acetyl glucosamine and yeast extract were found to stimulate growth. Tomato juice, free fatty acids and lyophilized beer solids (all of which are found in several media formulations for lactic acid bacteria) were inhibitory.

In comparative studies using in-process beer samples, Raka-Ray media gave higher colony counts for lactobacilli than Tomato Juice Agar, W-L Differential Agar and Universal Beer Agar, with larger colonies developing after 2-4 days of anaerobic incubation.[1,2]

Raka-Ray No. 3 Medium yields larger lactic acid bacterial colonies than Universal Beer Agar.[3] Raka-Ray No. 3 Medium also suppressed the growth of non-lactic acid, facultative bacteria, such as *Aerobacter aerogenes* and *Flavobacterium proteus* that are often associated with lactic beer spoilage organisms.[3]

Raka-Ray No. 3 Medium is also recommended by the 'European Brewing Congress Analytical Microbiologica' for enumeration of lactobacilli and pediococci.[4] The agar may be made more selective by the addition of 3 g of 2-phenylethanol and 7 mg

of cycloheximide dissolved in a small quantity of acetone per liter of medium before autoclaving. Yeasts and gram-negative bacteria are suppressed, facilitating enumeration of the lactic bacterial flora.

Principles of Procedure

Polysorbate 80, liver concentrate, maltose and other sugars, N-acetyl glucosamine and yeast extract stimulate the growth of lactobacilli. The optional addition of cycloheximide provides increased selectivity against yeasts and gram-negative bacteria.

Formula

Difco™ Raka-Ray No. 3 Medium

Approximate Formula* Per Liter		
Yeast Extract	5.0	g
Tryptone	20.0	g
Liver Concentrate	1.0	g
Maltose	10.0	g
Fructose	5.0	g
Glucose	5.0	g
Betaine Hydrochloride	2.0	g
Diammonium Citrate	2.0	g
Potassium Aspartate	2.5	g
Magnesium Sulfate	0.98	g
Manganese Sulfate	0.42	g
Dipotassium Phosphate	2.0	g
N-Acetyl Glucosamine	0.5	g
Potassium Glutamate	2.5	g
Agar	16.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 74.9 g of the powder in 1 L of purified water containing 10 mL polysorbate 80. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. To increase selectivity, add 3 g 2-phenylethanol and 7 mg cycloheximide per liter prior to boiling.
3. Autoclave at 121°C for 15 minutes. Avoid overheating, which will cause a softer medium.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Overlay Technique for Enumeration of Lactic Acid Bacteria

1. Inoculate 0.1 mL of the beer sample onto well-dried plates containing 15-20 mL Raka-Ray No. 3 Medium. Five replicates of each sample are recommended.
2. Spread over the surface of the medium using a sterile glass rod.
3. Overlay the surface with 4 mL of the molten sterilized medium cooled to 50°C.
4. Incubate plates at 27-30°C in an anaerobic (H_2/CO_2) atmosphere.

Expected Results

Lactobacilli are visible after 48 hours incubation as smooth, moist colonies that are 1 mm in diameter. Incubate the medium for a total of 7 days to allow development of slow-growing *Pediococcus* strains.

If the number of colonies on each plate exceeds 300, the sample should be diluted 1:10 in sterile physiological saline and retested.

References

1. Saha, Sondag and Middlekauff. 1974. An improved medium for the selective culturing of lactic acid bacteria. Proceedings of the American Society of Brewing Chemists. 9th Congress, p. 9.
2. VanKeer, Van Melkebeke, Vertriest, Hoozee and Van Schoonenberghe. 1983. J. Inst. Brewing *89*:360.
3. Report of the Technical Subcommittee. 1976. Microbiological Controls. J. Am. Soc. Brewing Chemists *34*:93.
4. European Brewing Congress Analytica Microbiologica. 1981. J. Inst. Brewing *87*:314.

Availability

Difco™ Raka-Ray No. 3 Medium

Cat. No. 218671 Dehydrated – 500 g

Rapid Fermentation Medium
Rapid Fermentation Medium with Carbohydrates

Intended Use

Rapid Fermentation Medium and Rapid Fermentation Medium with Carbohydrates provide a rapid method for the differentiation of *Neisseria* species isolated from clinical specimens.

Summary and Explanation

The occurrence of *Neisseria gonorrhoeae* in extra-genitourinary sites has made it important to differentiate this organism from other *Neisseria* species.[1,2] The isolation of *N. meningitidis* and *N. lactamica* from genitourinary sites further indicates the need for differentiation of these species.[1-3]

The classical method for the differentiation of *Neisseria* species is based upon determination of their carbohydrate utilization patterns. Pathogenic *Neisseria* are extremely fastidious organisms in both their growth and metabolic activities, thereby requiring an enriched culture medium. The conventional enriched medium for the determination of carbohydrate utilization is Cystine **Trypticase**™ Agar Medium (**CTA Medium**™) containing 0.5 to 1% carbohydrate.[3] The test battery includes dextrose, lactose, maltose and sucrose, and an identification can usually be made in 24 hours.

Rapid Fermentation Medium is a modification of the standard **CTA Medium**. The modified formula includes an increased agar and carbohydrate content that, when exposed to a large number of organisms, exhibits an acid shift by the phenol red indicator. An identification often can be made within 4 hours.

Principles of the Procedure

Rapid Fermentation Medium produces rapid results in detecting acid production from dextrose, maltose, sucrose and other carbohydrates. Although much of the literature refers to fermentation patterns for *N. gonorrhoeae*, it has been shown that this species metabolizes dextrose by strictly aerobic mechanisms; i.e., by a combination of the Entner-Doudoroff and pentose phosphate pathways. The utilization of dextrose by *N. gonorrhoeae*, as indicated by an acid change in the pH indicator present in the medium, is due to the production of acetic acid and small amounts of lactic acid.[4] The negative carbohydrate test is the result of the deamination of the peptone in the absence of any utilizable carbohydrate.

Procedure

1. Remove a full loopful of fresh colony growth from the surface of a Chocolate II Agar plate or slant. A large inoculum must be used in order to obtain a rapid reaction.
2. Deposit the inoculum below the surface of the medium and mix well.
3. Repeat for each tube to be inoculated. Inoculate a control tube (no carbohydrate) with *Neisseria* sp.
4. Incubate all tubes at 35 ± 2°C in an aerobic atmosphere without carbon dioxide. Observe periodically after 4 hours for reactions noted below. Continue incubation overnight if necessary. A few strains may require incubation for up to 48-72 hours.

Expected Results

After incubation, compare the reactions produced by the unknown isolates with those produced by known control organisms. Negative reactions are red. A positive reaction is indicated by a change of the phenol red indicator from red to yellow. The control cultures should produce results as shown in the table. If the results with the control cultures do not agree with those in the table, review the procedure, check the control cultures by Gram-staining and performing the oxidase test and repeat the fermentation test if necessary. The control tube (no carbohydrate) should be negative (red).

CONTROL ORGANISMS	ATCC™	PRODUCTION OF ACID FROM:
Neisseria gonorrhoeae	43070	Dextrose only
Neisseria meningitidis	13090	Dextrose and Maltose
Neisseria lactamica	23970	Dextrose, Maltose and Lactose
Neisseria sicca	29193	Dextrose, Maltose and Sucrose

If no positive carbohydrate reactions are observed within 4 hours, the tubes may be incubated overnight or longer to allow a positive reaction to develop.

References

1. Flynn and Waitkins. 1972. J. Clin. Pathol. 25:525.
2. Morse and Bartenstein. 1976. J. Clin. Microbiol. 3:8.
3. Knapp and Koumans. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Morse, Stein and Hines. 1974. J. Bacteriol. *120*:702.

Availability

BBL™ Rapid Fermentation Medium and Rapid Fermentation Medium with Carbohydrates

Cat. No.	221890	Prepared Tubes (K Tubes) – Pkg. of 10*
	221891	Prepared Tubes, Dextrose – Pkg. of 10*
	221893	Prepared Tubes, Lactose – Pkg. of 10*
	221894	Prepared Tubes, Maltose – Pkg. of 10*
	221895	Prepared Tubes, Sucrose – Pkg. of 10*

**Store at 2-8°C.*

Rapid Urea Broth

Intended Use

Rapid Urea Broth is used for the presumptive identification of *Helicobacter pylori* in gastric antral biopsy specimens.

Summary and Explanation

The presence of small, curved and S-shaped bacilli in antral biopsy specimens was first reported by Warren and Marshall in 1983.[1] Subsequently, other investigators observed an association between this organism, now known as *Helicobacter pylori,* and gastritis.[2-4] However, isolating and identifying the organism on primary media may require up to 7 days, delaying treatment.[5]

McNulty developed a rapid diagnostic test for *Helicobacter*-associated gastritis. Noting that the rapid hydrolysis of urea is characteristic of *H. pylori*, he placed biopsy specimens in Christensen's 2% urea broth and observed a color change. Depending on the number of organisms present in the specimen, positive results could be observed in less than one hour.[6]

Principles of the Procedure

The enzyme urease catalyzes the hydrolysis of urea to ammonium and bicarbonate ions. Because urease is not a human enzyme, its activity in the gastric mucosa is due primarily to the presence of *H. pylori*.[7] The preformed urease enzyme present in biopsy specimens renders the urease broth alkaline and changes the phenol red indicator in the medium to a pink-red color.

Procedure

After the specimen is obtained by antral biopsy, it should be placed immediately into Rapid Urea Broth. If the specimen is transported in saline to the laboratory, it should be transferred immediately to Rapid Urea Broth. Place vials in an incubator at 35 ± 2°C and observe for development of a red color within 1 hour. If negative, continue incubation and observe periodically for up to 4 hours.

Expected Results

The presence of urease is indicated by an intense pink-red color throughout the broth. A negative reaction is no color change; i.e., the broth medium remains yellowish-orange. Results should be confirmed by Gram-staining and subculturing to an appropriate medium; e.g., Brucella Agar with 5% sheep blood or 5% horse blood.[7-10]

Limitation of the Procedure

Urea test media rely on demonstration of alkalinity; hence they are not specific for urease. The utilization of peptones by contaminating organisms or other proteins in the medium may raise the pH to alkalinity due to protein hydrolysis and release of excessive amino acid residues, resulting in false positive reactions.[11]

References

1. Warren and Marshall. 1983. Lancet *i:* 1273.
2. Rauws, Langenberg, Houthoff, Zaneen and Tytgat. 1988. Gastroenterology *94*:33.
3. Marshall, Armstrong, McGechie and Glancy. 1985. Med. J. Aust. *142*:436.
4. Buck, Gourley, Lee, Subramanyam, Latimer and DiNuzzo. 1986. J. Infect. Dis. *153*:664.
5. Czinn and Carr. 1987. J. Pediatr. *110*:569.
6. McNulty and Wise. 1985. Lancet *i*: 1443.
7. Bates. 1987. Lab. Management 25(11):27.
8. Coudron and Kirby. 1989. J. Clin. Microbiol. *27*:1527.
9. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
10. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
11. Westblom, Madan, Kemp and Subik. 1988. J. Clin. Microbiol. *26*:1393.

Availability

BBL™ Rapid Urea Broth

Cat. No. 298330 Prepared Vial, 0.5 mL – Pkg. of 10*

**Store at 2-8°C.*

Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification Novobiocin Antimicrobic Supplement

Intended Use

Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification is used with Novobiocin Antimicrobic Supplement in rapidly detecting motile *Salmonella* in feces and food products.

Summary and Explanation

MSRV Medium, Semisolid Modification is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella* in feces and food products. The original work on MSRV medium showed that a semi-solid medium in Petri dishes could be used as a rapid and sensitive means of isolating motile *Salmonella* from food products following pre-enrichment or selective enrichment.[1,2] The semisolid medium allows motility to be detected as halos of growth around the original point of inoculation.

The medium is recommended by the European Chocolate Manufacturer's Association. A collaborative study performed with support of the American Cocoa Research Institute and the Canadian Chocolate Manufacturer's Association resulted in first action adoption of the MSRV method by AOAC International.[3]

MSRV Medium, Semisolid Modification may be used as a plating medium for isolating *Salmonella* spp. (other than *Salmonella* Typhi and *Salmonella* Paratyphi A) from stool specimens with high sensitivity and specificity.[4,5]

Principles of the Procedure

Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification contains peptones as carbon and nitrogen sources for general growth requirements. Magnesium chloride raises the osmotic pressure in the medium. Novobiocin (Novobiocin Antimicrobic Supplement) and malachite green inhibit organisms other than *Salmonella*. The low pH of the medium, combined with the novobiocin, malachite green and magnesium chloride, helps to select for highly resistant *Salmonella* spp. Agar is the solidifying agent.

Formulae

Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification

Approximate Formula* Per Liter	
Tryptose	4.59 g
Casein Hydrolysate (acid)	4.59 g
Sodium Chloride	7.34 g
Monopotassium Phosphate	1.47 g
Magnesium Chloride (anhydrous)	10.93 g
Malachite Green Oxalate	37.0 mg
Agar	2.7 g

Difco™ Novobiocin Antimicrobic Supplement

Formula per 10 mL Vial	
Sodium Novobiocin	20.0 mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification

1. Suspend 31.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE. Cool to 50°C.
3. Aseptically add 10 mL of Novobiocin Antimicrobic Supplement. Mix thoroughly.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Novobiocin Antimicrobic Supplement

Rehydrate with 10 mL sterile purified water. Mix well.

User Quality Control

Identity Specifications

Difco™ Rappaport-Vassiliadis Medium, Semisolid Modification

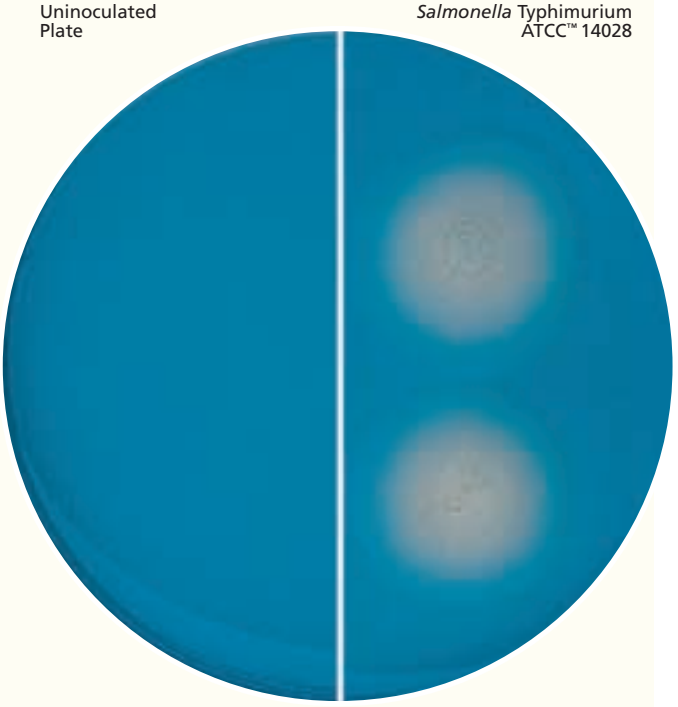
Dehydrated Appearance:	Pale green, free-flowing, homogeneous.
Solution:	3.16% solution, soluble in purified water upon boiling. Solution is blue, clear to slightly opalescent.
Prepared Appearance:	Blue, slightly opalescent, semisolid.
Reaction of 3.16% Solution at 25°C:	pH 5.2 ± 0.2

Cultural Response

Difco™ Rappaport-Vassiliadis Medium, Semisolid Modification

Prepare the medium per label directions. Inoculate and incubate at 42 ± 0.5°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HALO/ MOTILITY
Citrobacter freundii	8090	10^3-2×10^3	Marked inhibition	–
Proteus mirabilis	9240	10^3-2×10^3	None	–
Pseudomonas aeruginosa	27853	10^3-2×10^3	None	–
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10^2-10^3	Good	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	+
Salmonella senftenberg (NCTC 10384)		10^2-10^3	Good	+



Procedure[3,6]

Pre-enrichment

1. Add 25 g of cocoa or chocolate to 225 mL of sterile reconstituted nonfat dry milk with 0.45 mL of a 1% aqueous brilliant green dye solution; mix well.[6]
2. Incubate at 35°C for 20 ± 2 hours.[3]

Selective Enrichment[3]

3. Inoculate 10 mL Tetrathionate Broth (prewarmed to 35°C) with 1 mL of the pre-enrichment culture.
4. Incubate at 35°C for 8 ± 0.5 hours.

Motility Enrichment on MSRV, Semisolid Modification[3]

5. After selective enrichment incubation, mix the broth culture. Inoculate 3 drops at separate spots on an MSRV plate.
6. Incubate at 42 ± 0.5°C for 16 ± 0.5 hours.

Expected Results

Positive: Growth of migrated cells is visible as a gray-white, turbid zone extending out from the inoculated drop. Test sample is considered presumptively positive for motile *Salmonella*.

Negative: Medium remains blue-green around the drops, with no gray-white, turbid zone extending out from the drop. Test sample is considered negative for motile *Salmonella*.

To confirm a presumptive identification of *Salmonella*:[3]

Rapid serologic confirmation

1. Inoculate M Broth with growth from migration edge on MSRV, Semisolid Modification plate.
2. Incubate at 35°C for 4-6 hours (until turbid). M Broth culture can be held for up to 24 hours at 35°C.
3. Test with *Salmonella* O and H antisera.

Culture confirmation

1. Transfer a loopful of growth from the migration edge on MSRV, Semisolid Modification plate onto Hektoen Enteric Agar and streak for isolation.
2. Incubate at 35°C for 24 ± 2 hours.
3. From colonies of Hektoen agar that show colony appearance typical of *Salmonella* (green colonies with black centers), perform biochemical tests to confirm the identification.

Limitation of the Procedure

The combination of malachite green, magnesium chloride and a low pH may inhibit certain *Salmonella*, such as *Salmonella* Typhi and *Salmonella* Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. DeSmedt, Bolderdijk, Rappold and Lautenschlaeger. 1986. J. Food Prot. *49*:510.
2. DeSmedt and Bolderdijk. 1987. J. Food Prot. *50*:658.
3. DeSmedt, Bolderdijk and Milas. 1994. J. AOAC Int. *77*:365.
4. Dusch and Altwegg. 1995. J. Clin. Microbiol. *33*:802.
5. Aspinall, Hindle and Hutchinson. 1992. Eur. J. Clin. Microbiol. Infect. Dis. *11*:936.
6. Andrews and Hammack. 2001. *In* FDA bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification

CCAM

Cat. No. 218681 Dehydrated – 500 g

Difco™ Novobiocin Antimicrobic Supplement

AOAC

Cat. No. 231971 Vial – 6 × 10 mL*

*Store at 2-8°C.

Rappaport-Vassiliadis R10 Broth

Intended Use

Rappaport-Vassiliadis R10 Broth is used for selectively enriching *Salmonella* from meat and dairy products, feces and sewage-polluted water.

Summary and Explanation

Rappaport et al.[1] formulated an enrichment medium for *Salmonella* that was modified by Vassiliadis et al.[2] The Rappaport formulation, designated R25/37°C, recommended incubation at 37°C; the Vassiliadis modification, designated R10/43°C, had a reduced level of malachite green and recommended incubation at 43°C. Later work by Peterz showed that incubation at 41.5° ± 0.5°C for 24 hours improved recovery of *Salmonella* spp.[3]

Rappaport-Vassiliadis R10 Broth is a selective enrichment medium that is used following pre-enrichment of the specimen in a suitable pre-enrichment medium. It has gained approval for use in analyzing milk and milk products,[4] raw flesh foods, highly contaminated foods and animal feeds.[5]

User Quality Control

Identity Specifications

Difco™ Rappaport-Vassiliadis R10 Broth

Dehydrated Appearance:	Pale green to green, free-flowing, homogeneous.
Solution:	2.66% solution, soluble in purified water upon gentle heating. Solution is blue, clear.
Prepared Appearance:	Blue, clear.
Reaction of 2.66% Solution at 25°C:	pH 5.1 ± 0.2

Cultural Response

Difco™ Rappaport-Vassiliadis R10 Broth

Prepare the medium per label directions. Inoculate and incubate at 41.5 ± 0.5°C for 18-48 hours. Subculture to Brilliant Green Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-2×10^3	Marked inhibition
Proteus mirabilis	9240	10^3-2×10^3	Marked inhibition
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good

This medium selectively enriches for salmonellae because bacteria, including other intestinal bacteria, are typically inhibited by malachite green, high osmotic pressure and/or low pH. *Salmonella* Typhi and *S.* Paratyphi A are sensitive to malachite green and may be inhibited.

Principles of the Procedure

Rappaport-Vassiliadis R10 Broth contains peptone as the carbon and nitrogen source for general growth requirements. Magnesium chloride raises the osmotic pressure in the medium. Malachite green is inhibitory to organisms other than salmonellae. The low pH of the medium, combined with the presence of malachite green and magnesium chloride, helps to select for the highly resistant *Salmonella* spp.

Formula

Difco™ Rappaport-Vassiliadis R10 Broth

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	4.54 g
Sodium Chloride	7.2 g
Monopotassium Phosphate	1.45 g
Magnesium Chloride (anhydrous)	13.4 g
Malachite Green Oxalate	36.0 mg

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 26.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense 10 mL amounts into suitable containers.
4. Autoclave at 116°C (10 psi pressure) for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure and Expected Results

Water and Sewage Samples

For isolating *Salmonella* (other than *S.* Typhi) from water and associated materials, such as sewage liquor, sewage sludge, digested sludge and pressed sludge cake:

1. Concentrate the sample by filtering it through a plug of sterile absorbent cottonwool inserted in the neck of a large sterile funnel or through a Whatman No. 17 absorbent pad.

Pre-enrichment

2. Using aseptic technique, transfer the cottonwool plug or the pad to 100 mL of a suitable pre-enrichment medium such as Buffered Peptone Water.
3. Incubate at 37 ± 0.5°C for 18-24 hours.

Selective Enrichment

4. Inoculate 10 mL of Rappaport-Vassiliadis R10 Broth with 0.1 mL of the pre-enrichment culture. Inoculate 10 mL of Muller-Kauffman Tetrathionate Broth with 1 mL of the pre-enrichment culture.
5. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C. Incubate Muller- Kauffman Tetrathionate Broth at 42 ± 1°C for 48 hours.

Expected Results

6. After incubation, subculture both selective enrichment broths to Brilliant Green Agar and XLD Agar. Incubate at 35 ± 2°C for 18-24 hours.
7. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

Milk and Foods

For isolating *Salmonella* (other than *S.* Typhi) from milk and milk products,[4] raw flesh foods, highly contaminated foods and animal feeds:[5]

Pre-enrichment

1. Add 25 g or a 25 mL sample of the specimen to 225 mL of pre-enrichment medium. Consult appropriate references for the type of product being tested.[4,5]
2. Incubate at 35 ± 2°C for 20-24 hours[5] or at 37°C for 16-20 hours,[4] depending on the referenced procedure being followed.

Selective Enrichment

3. Inoculate 10 mL of Rappaport-Vassiliadis R10 Broth with 0.1 mL of pre-enrichment culture. Inoculate 10 mL of another selective enrichment medium such as Tetrathionate Broth or Selenite Cystine Broth with the recommended amount of pre-enrichment culture.[4,5]
4. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C[4] for 24 ± 2 hours or at 42 ± 0.5°C for 22-24 hours.[5] Incubate the other selective enrichment broths appropriately.

Expected Results

5. After incubation, subculture Rappaport-Vassiliadis R10 Broth and the other selective enrichment broths to selective agar media and incubate at 35 ± 2°C for 24 ± 2 hours[4] or for 18-24 hours.[5]
6. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.[4,5]

Limitation of the Procedure

The combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain *Salmonella*, such as *Salmonella* Typhi and *S.* Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. Rappaport, Konforti and Navon. 1956. J. Clin. Pathol. 9:261.
2. Vassiliadis, Trichopoulos, Kalandidi and Xirouchaki. 1978. J. Appl. Bacteriol. *44*:233.
3. Peterz, Wiberg and Norberg. 1989. J. Appl. Bacteriol. *66*:523.
4. International Organization for Standardization. 2001. Milk and milk products – detection of *Salmonella*. ISO 6785/IDF 93:2001. ISO, Geneva, Switzerland.
5. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Difco™ Rappaport-Vassiliadis R10 Broth

IDF ISO USDA

Cat. No. 218581 Dehydrated – 500 g

Europe

Cat. No. 257257 Prepared Tubes, 10 mL – Ctn. of 50

Rappaport Vassiliadis Salmonella (RVS) Soy Broth

Intended Use

Rappaport Vassiliadis Salmonella (RVS) Soy Broth is used for selectively enriching *Salmonella* in food and environmental samples.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Rappaport et al.[4] formulated an enrichment medium for *Salmonella* that included very high amounts of malachite green and magnesium chloride as inhibitors. The original Rappaport medium was developed for the enrichment of *S. paratyphi* and other serotypes that were known to be relatively resistant to brilliant green. In addition, magnesium chloride was found to counteract the toxic effect of the dye for *Salmonella*.[5] Vassiliadis et al. modified the formulation by reducing the concentration of the malachite green to one third.[6]

Van Schothorst and Renaud reported that using soy peptone instead of animal peptone improved recovery rates of *Salmonella*.[7] Similar results were obtained in several other studies.[8-11]

Vassiliadis et al. recommended incubation of RV media at 43°C for maximum selectivity.[6] Any deviation above 43°C may be lethal for *Salmonella*. Later, work by Peterz showed that incubation at 41.5 ± 0.5°C for 24 hours improved recovery of *Salmonella* spp.[12]

RVS Soy Broth is a selective enrichment medium that is used following pre-enrichment of a sample in a suitable pre-enrichment medium. It has gained approval for use in analyzing milk

and milk products,[13] food,[14,15] animal feed,[15] and nonsterile pharmaceutical products.[1] This medium selectively enriches for salmonellae because bacteria, including other intestinal bacteria, are typically inhibited by malachite green, high osmotic pressure and/or low pH. *S.* Typhi and *S.* Paratyphi A are sensitive to malachite green and may be inhibited.

Principles of the Procedure

RVS Soy Broth contains soy peptone as the carbon and nitrogen source for general growth requirements. Magnesium chloride raises the osmotic pressure in the medium. Sodium chloride maintains osmotic balance. Dipotassium phosphate and potassium dihydrogen phosphate are buffering agents. Malachite green is inhibitory to organisms other than salmonellae. The low pH of the medium, combined with the presence of malachite green and magnesium chloride, helps to select for the highly resistant *Salmonella* spp.

Formula

Difco™ RVS Soy Broth

Approximate Formula* Per Liter		
Soy Peptone	4.5	g
Magnesium Chloride (anhydrous)	13.5	g
Sodium Chloride	9.0	g
Dipotassium Phosphate	0.03	g
Potassium Dihydrogen Phosphate	1.45	g
Malachite Green	36.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

NOTE: Formula is further adjusted from the *USP* formulation as follows. Since magnesium chloride hexahydrate contains too much water to be effectively used in the manufacture of dehydrated culture media, magnesium chloride anhydrous (without water) is substituted. The actual amount of magnesium chloride (minus water) is the same. However, the use of the anhydrous magnesium requires slight adjustments in the rest of the formulation. None of these slight changes affect performance, as is indicated on the Certificate of Analysis, which shows that harmonized *USP/EP/JP* growth promotion criteria are met per requirements for the Microbiological Examination of Nonsterile Products.

Directions for Preparation from Dehydrated Product

1. Suspend 28.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense 10 mL amounts into suitable containers.
4. Autoclave at 115°C (10 psi pressure) for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

Follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[1,13-15]

User Quality Control

Identity Specifications

Difco™ RVS Soy Broth

Dehydrated Appearance:	Pale green to green, free-flowing, homogeneous.
Solution:	2.85% solution, soluble in purified water upon gentle heating. Solution is blue, clear.
Prepared Appearance:	Blue, clear.
Reaction of 2.85% Solution at 25°C:	pH 5.2 ± 0.2

BBL™ RVS Soy Broth (prepared)

Appearance:	Blue and clear.
Reaction at 25°C:	pH 5.2 ± 0.2

Cultural Response

Difco™ RVS Soy Broth

Prepare the medium per label directions. Inoculate and incubate at 30-35°C for 24 hours. After incubation, subculture to XLD Agar plates and incubate at 30-35°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	<100	Growth
Staphylococcus aureus	6538	>100	No growth

BBL™ RVS Soy Broth (prepared)

Inoculate and incubate at 30-35°C for 18-24 hours. After incubation, subculture to XLD Agar plates and incubate at 30-35°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10-100	Growth
Staphylococcus aureus	6538	>100	No growth

Procedure

Refer to appropriate references for details on test methods using RVS Soy Broth.[1,13-15] Inoculate tubes with the test sample and incubate as instructed in appropriate references.[1,13-15]

Expected Results

Examine selective plates for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and/or serological tests as directed in appropriate references.

Limitation of the Procedure

The combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain *Salmonella*, such as *S.* Typhi and *S.* Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.

References

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14. United Stated Department of Agriculture. 2008. Microbiology laboratory guidebook, online. MLG 4.04, Isolation and identification of *Salmonella* from meat, poultry and egg products. USDA, Food Safety and Inspection Service, Office of Public Health Science, Athens, Ga.
15. International Organization for Standardization. 2002. Microbiology of food and animal feeding stuffs – horizontal method for the detection of *Salmonella* spp. ISO 6579, 2002-07-15. International Organization for Standardization, Geneva, Switzerland.

Availability

Difco™ RVS Soy Broth

CCAM EP ISO JP USDA USP

Cat. No. 214943 Dehydrated – 500 g†

BBL™ RVS Soy Broth

CCAM EP ISO JP USDA USP

Cat. No. 215199 Prepared Tubes, 10 mL – Pkg. of 10†

† QC testing performed according to USP/EP/JP performance specifications.

Regan-Lowe Charcoal Agar
Regan-Lowe Charcoal Agar without Cephalexin

Intended Use

Regan-Lowe Charcoal Agar is a selective medium used for isolation of *Bordetella pertussis* from clinical specimens. Regan-Lowe Charcoal Agar without Cephalexin is used for the cultivation of *B. pertussis* from clinical specimens and for subcultures of the bacterium.

Summary and Explanation

Regan-Lowe Charcoal Agar plates are used in clinical laboratories for the isolation of *Bordetella pertussis*, the etiologic agent of whooping cough, from nasopharyngeal swabs and other sources of pharyngeal exudate. This medium was developed by Regan and Lowe as a transport medium for whooping cough specimens, but proved useful as an enrichment medium for the selective isolation of *B. pertussis* and *B. parapertussis*. It consists of charcoal agar as a basal medium supplemented with cephalexin to inhibit bacteria indigenous to the nasopharynx and defibrinated horse blood to support the growth of *Bordetella* species.[1-3]

Use of the medium without cephalexin in parallel with Regan-Lowe Charcoal Agar is recommended, since a few strains (<10%) of *B. pertussis* will not grow on selective plates; also the nonselective medium is used for subcultures to obtain a larger amount of growth for additional testing, such as agglutination or immunofluorescence testing.[3,4]

The medium in 10 mL prepared tubes (deeps) with screw-caps offers a longer shelf-life than the pre-poured plated medium.

To prepare the medium from the agar base, 10% horse blood is added and cephalexin can be added to achieve selectivity.

Principles of the Procedure

Beef extract and enzymatic digest of gelatin provide the amino acids and other complex nitrogenous substances necessary

User Quality Control

Identity Specifications

BBL™ Regan-Lowe Charcoal Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.1% solution, soluble in purified water upon boiling. Solution is charcoal black, homogeneous, opaque.
Prepared Appearance:	Charcoal black, homogeneous, opaque.
Reaction of 5.1% Solution at 25°C:	pH 7.4 ± 0.2

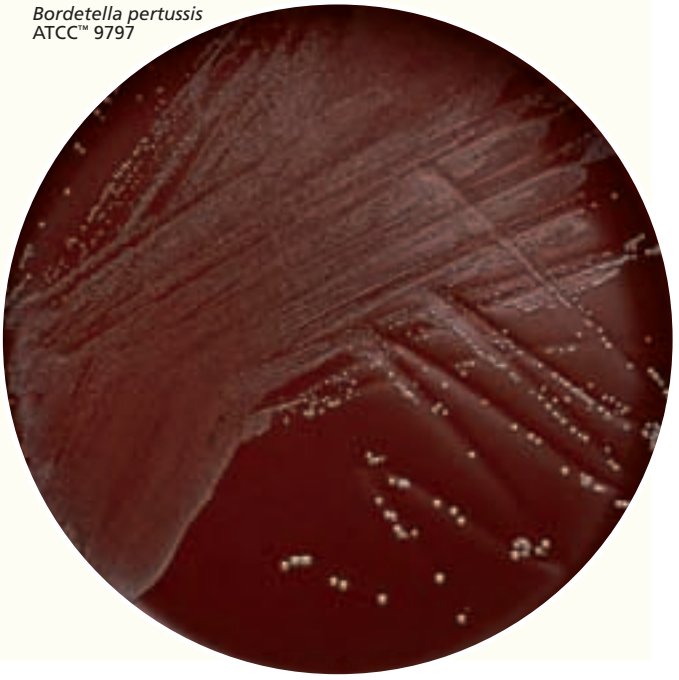
Cultural Response

BBL™ Regan-Lowe Charcoal Agar Base

Prepare the medium per label directions. Inoculate with fresh broth cultures diluted 1:10 and incubate at 35 ± 2°C for 7 days.

ORGANISM	ATCC™	RECOVERY
Bordetella pertussis	9797	Good
Bordetella parapertussis	15311	Good

Bordetella pertussis
ATCC™ 9797



to support bacterial growth. Sodium chloride maintains the osmotic equilibrium. Defibrinated horse blood supplies nutrients required for the cultivation of *Bordetella* species. Nicotinic acid is a vitamin that promotes growth. Charcoal and starch neutralize substances toxic to *Bordetella* species, such as fatty acids and peroxides. Cephalexin is a cephalosporin antibiotic that inhibits most normal flora of the nasopharynx.

Formula

BBL™ Regan-Lowe Charcoal Agar Base

Approximate Formula* Per Liter

Beef Extract	10.0	g
Pancreatic Digest of Casein	10.0	g
Soluble Starch	10.0	g
Sodium Chloride	5.0	g
Charcoal	4.0	g
Niacin	0.01	g
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 51 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.
4. For preparation of blood plates, add 10% sterile, defibrinated horse blood to sterile agar which has been previously melted and cooled to 45-50°C.
5. For selective isolation of *B. pertussis* and *B. parapertussis*, add 40 µg of cephalexin per mL.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) in a moist chamber at 35°C for 7 days. Colonies of *B. pertussis* may not be visible without the aid of a microscope for 2-4 days. Plates may be discarded as negative after 7 days of incubation.

Expected Results

Examine the plates daily with and without a dissecting microscope (oblique illumination) to detect the presence of *B. pertussis*. *B. pertussis* produces small, domed, glistening, white to gray colonies. To prevent overgrowth by spreading colonies or molds, use a sterile scalpel or needle to remove the portions of the agar that contain these contaminants.

References

1. Regan and Lowe. 1977. J. Clin. Microbiol. 6:303.
2. Sneed. 1992. *In* Isenberg (ed.), Clinical microbiology procedure handbook, vol. 1. American Society for Microbiology, Washington, D.C.
3. Marcon. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Regan-Lowe Charcoal Agar Base

Cat. No. 298123 Dehydrated – 500 g

BBL™ Regan-Lowe Charcoal Agar

BS12 CMPH2 MCM9

Cat. No. 297883 Prepared Plates – Pkg. of 10*
297855 Prepared Tubes (Deeps), 10 mL – Pkg. of 10*

BBL™ Regan-Lowe Charcoal Agar without Cephalexin

Cat. No. 298326 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Reinforced Clostridial Medium

Intended Use

Reinforced Clostridial Medium is used for cultivating and enumerating clostridia, other anaerobes, and other species of bacteria from foods and clinical specimens.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Reinforced Clostridial Medium is a semisolid medium formulated by Hirsch and Grinstead.[4] Their work demonstrated that the medium outperformed other media in supporting growth of clostridia from small inocula and produced higher viable cell counts.[4] Barnes and Ingram[5] used the medium to dilute vegetative cells of *Clostridium perfringens*. Barnes et al.[6] used a solid (agar) version of the medium to enumerate clostridia in food.

Reinforced Clostridial Medium is a nonselective enrichment medium and grows various anaerobic and facultative bacteria when incubated anaerobically.[7] This medium has been used to detect clostridia, bifidobacteria and other anaerobes in food products[8-11] and fecal samples.[12] Reinforced Clostridial Medium is listed in the *USP* as the recommended medium for the isolation of *Clostridium* sp. from nonsterile pharmaceutical products.[1]

Principles of the Procedure

Reinforced Clostridial Medium contains peptone and beef extract as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Sodium chloride maintains the osmotic balance. In low concentrations, soluble starch detoxifies metabolic by-products. Cysteine HCl is the reducing agent. Sodium acetate acts as a buffer. The small amount of agar makes the medium semisolid.

User Quality Control

Identity Specifications

Difco™ Reinforced Clostridial Medium

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is medium amber, slightly opalescent with dark particles and flocculation when hot.
Prepared Appearance:	Upon cooling, medium amber and becomes more opalescent.
Reaction of 3.8% Solution at 25°C:	pH 6.8 ± 0.2

BBL™ Reinforced Clostridial Medium (prepared)

Appearance:	Light to medium amber and opalescent with particles.
Reaction at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Reinforced Clostridial Medium

Prepare the medium per label directions. Inoculate and incubate tubes with caps tightened at 35 ± 2°C for 18-48 hours. Inoculate 100 mL bottles with *C. sporogenes* cultures and incubate with caps tightened at 30-35°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	23745	30-300	Good
Clostridium botulinum	3502	30-300	Good
Clostridium perfringens	13124	30-300	Good
Clostridium sporogenes	19404	<100	Growth
Clostridium sporogenes	11437	<100	Growth

BBL™ Reinforced Clostridial Medium (prepared)

Inoculate and incubate bottles with caps tightened at 30-35°C for up to 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Clostridium perfringens	13124	10-100	Fair to good
Clostridium sporogenes	19404	<100	Growth
Clostridium sporogenes	11437	<100	Growth

Formula

Difco™ Reinforced Clostridial Medium

Approximate Formula* Per Liter		
Peptone	10.0	g
Beef Extract	10.0	g
Yeast Extract	3.0	g
Dextrose	5.0	g
Sodium Chloride	5.0	g
Soluble Starch	1.0	g
Cysteine HCl	0.5	g
Sodium Acetate	3.0	g
Agar	0.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions[13]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing *C. botulinum* or *C. tetani* or their toxins.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of these organisms and for activities with a high potential for aerosol or droplet production, and those involving production quantities of toxin.

Directions for Preparation from Dehydrated Product

1. Suspend 38 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Refer to *USP* General Chapter <62> for details on the examination of nonsterile products and the isolation of clostridia using Reinforced Clostridial Medium.[1]

Expected Results

After appropriate incubation time and temperature, subculture each tube or bottle to two Columbia Agar plates. Incubate under both aerobic and anaerobic conditions for 48 hours at 30-35°C to confirm the presence of anaerobic growth. After incubation of these plates, if isolates grow anaerobically only (with or without endospores) and are catalase negative, this indicates the presence of *Clostridium* sp.[1] Perform other confirmatory biochemical testing as necessary.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
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5. Barnes and Ingram. 1956. J. Appl. Bacteriol. *19*:117.
6. Barnes, Despaul and Ingram. 1963. J. Appl. Bacteriol. *26*:415.
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8. Mead. 1995. Principles involved in the detection and enumeration of clostridia in foods. *In* Corry, J.E.L., et al. (eds.), Culture media for food microbiology. Elsevier Science B.V. Amsterdam, The Netherlands.
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11. Health Canada. The compendium of analytical methods, online. Food Directorate, Health Products and food Branch, Health Canada, Ottawa, Ontario Canada.
12. Hartemink and Rombouts. 1999. J. Microbiol. Methods. *36*:181.
13. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.

Availability

Difco™ Reinforced Clostridial Medium

CCAM EP JP USP

Cat. No. 218081 Dehydrated – 500 g†

BBL™ Reinforced Clostridial Medium

CCAM EP JP USP

Cat. No. 215192 Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10†

Europe

CCAM EP

Cat. No. 254548 Prepared Plates – Pkg. of 20*

** Store at 2-8°C.*

† QC testing performed according to USP/EP/JP performance specifications.

Riboflavin Assay Medium

Intended Use

Riboflavin Assay Medium is used for determining riboflavin concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For maintaining the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Riboflavin Assay Medium is a modification of the medium described by Snell and Strong.[1] It is recommended for use in the microbiological assay of riboflavin following the methodology outlined in the *Official Methods of Analysis of AOAC International*[2] using *Lactobacillus rhamnosus* ATCC™ 7469 as the test organism.

User Quality Control

Identity Specifications

Difco™ Riboflavin Assay Medium

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.4% solution (single strength) and 4.8% (double strength), soluble in purified water upon boiling. Solution is light to medium amber, clear, may have a slight precipitate.
Prepared Appearance:	Light amber, clear, may have a very slight precipitate.
Reaction of 2.4% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Riboflavin Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus rhamnosus* ATCC™ 7469 when prepared in single strength and supplemented with riboflavin. The medium should produce a standard curve when tested using a riboflavin reference standard at 0.0 to 125.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Principles of the Procedure

Riboflavin Assay Medium is free from riboflavin but contains all other nutrients and vitamins essential for the growth of *Lactobacillus rhamnosus* ATCC 7469. The addition of riboflavin in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Riboflavin Assay Medium

Approximate Formula* Per Liter		
Dextrose	20.0	g
Sodium Acetate	15.0	g
Vitamin Assay Casamino Acids	10.0	g
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
L-Asparagine	0.6	g
DL-Tryptophan	0.2	g
L-Cystine	0.2	g
Magnesium Sulfate USP	0.4	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Xanthine	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate (monohydrate)	20.0	mg
Sodium Chloride USP	20.0	mg
Pyridoxine Hydrochloride	4.0	mg
Pyridoxal Hydrochloride	4.0	mg
p-Aminobenzoic Acid	2.0	mg
Calcium Pantothenate	800.0	µg
Folic Acid	800.0	µg
Nicotinic Acid	800.0	µg
Thiamine Hydrochloride	400.0	µg
Biotin	1.0	µg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware, free from detergents and other chemicals, must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic esidues that might be present. Take precautions to keep sterilizing and cooling conditions uniform throughout assay.

Directions for Preparation from Dehydrated Product

1. Suspend 4.8 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the volume to 10 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

Procedure

Follow applicable assay procedures.[2] Levels of riboflavin used in the determination of the standard curve should be prepared according to this reference or according to the following procedure.

Stock Cultures

Stock cultures of *L. rhamnosus* ATCC 7469 are prepared by stab inoculation into 10 mL of Lactobacilli Agar AOAC. After 24-48 hours incubation at 35-37°C, the stock cultures are kept in the refrigerator. Transfers are made at monthly intervals in triplicate.

Inoculum

Inoculum for assay is prepared by subculturing a stock culture of *L. rhamnosus* ATCC 7469 into 10 mL of Lactobacilli Broth AOAC or Micro Inoculum Broth. Following incubation for 16-24 hours at 35-37°C, the culture is centrifuged under aseptic conditions and the supernatant liquid decanted. After washing 3 times with 10 mL sterile 0.85% saline, the cells are resuspended in 10 mL sterile 0.85% saline. The cell suspension is then diluted with sterile 0.85% saline, to a turbidity of 35-40% transmittance when read on the spectrophotometer at 660 nm. One drop of this latter suspension is then used to inoculate each of the assay tubes.

Riboflavin Assay Medium may be used for both turbidimetric and titrimetric determinations. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C, whereas titrimetric determinations are best made after 72 hours incubation at 35-37°C. Using Riboflavin Assay Medium, the most effective assay range is between 0.025 and 0.15 µg riboflavin.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation, which influence the standard curve readings, cannot be duplicated exactly from assay to assay. The standard curve is obtained by using Riboflavin USP Reference Standard or equivalent at levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 and 0.3 µg riboflavin per assay tube (10 mL).

The concentration of riboflavin required for the preparation of the standard curve may be prepared by dissolving 0.1 g of Riboflavin USP Reference Standard or equivalent in 1,000 mL of purified water by heating, giving a stock solution of 100 µg per mL. Dilute the stock solution by adding 1 mL to 999 mL purified water. Use 0.0, 0.25, 0.5, 0.75, 1, 1.5, 2 and 3 mL of the diluted stock solution per tube. Prepare the stock solution fresh daily.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average and use the results only if two-thirds of the values do not vary by more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.
5. Maintain pH below 7.0 to prevent loss of riboflavin.

References

1. Snell and Strong. 1939. Ind. Eng. Chem. *11*:346.
2. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Riboflavin Assay Medium

AOAC

Cat. No. 232510 Dehydrated – 100 g*

**Store at 2-8°C.*

Rice Extract Agar

Intended Use

Rice Extract Agar is used for promotion of chlamydospore formation by *Candida albicans* and *C. stellatoidea* as a means of differentiating them from other *Candida* species.

Summary and Explanation

Rice Extract Agar was developed by Taschdjian to aid in the identification of chlamydospore-producing species of *Candida* so as to differentiate these species from others within the *Candida* genus.[1] Later, Taschdjian recommended inclusion of polysorbate 80 and the use of a lower concentration of medium (13 g/L) to enhance the formation of chlamydospores.[2]

Rice Extract Agar with 2% dextrose may be used to promote chromogenesis and, therefore, is helpful in distinguishing *Trichophyton rubrum* from *T. mentagrophytes*.

Principles of the Procedure

The rice extract provides the nutrients required for the growth of *Candida* species. The addition of polysorbate 80 stimulates chlamydospore formation due to its content of oleic acids. Chlamydospore production is also favored by the use of a lower concentration, 13 g/L, although the medium can be prepared at a higher concentration (25 g/L).

The addition of 2% dextrose enhances chromogenesis in *T. rubrum*.

Formula

BBL™ Rice Extract Agar

Approximate Formula* Per Liter

White Rice, Extract from (solids)	5.0	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 25 g of the powder in 1 L of purified water. To promote chlamydospore formation, suspend 13 g of the powder in 1 L of purified water.
2. Add 10 mL polysorbate 80. Mix until a uniform suspension is obtained.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Dispense and autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. For use in the detection of chlamydospore formation. Inoculate the culture by cutting slits into the agar with an inoculating needle. Cover the inoculated slits with sterile coverslips. Seal the plates to avoid moisture loss and incubate at room temperature for 24-48 hours and up to 14 days before discarding as negative.

User Quality Control

Identity Specifications

BBL™ Rice Extract Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.3% solution, soluble in purified water upon boiling. Solution is pale, yellow to tan, moderately hazy to hazy.
Prepared Appearance:	Pale, yellow to tan, moderately hazy to hazy.
Reaction of 1.3% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

BBL™ Rice Extract Agar

Prepare the medium per label directions and test for chlamydospore production. Using fresh cultures, streak two parallel lines approximately 1.5 cm long and 1.0 cm apart. Make an S-shaped streak lightly back and forth across the two parallel streak lines. Place a coverslip over the streak marks. Incubate at 20-25°C for 3-5 days and examine microscopically.

ORGANISM	ATCC™	RECOVERY	CHLAMYDOSPORES
Candida albicans	10231	Good	+
Candida albicans	60193	Good	_

2. For use in the promotion of chromogenesis in *T. rubrum*. Streak-inoculate tubed medium slants. Tighten caps after inoculation and then loosen slightly. After incubation for 2-3 days, caps should be retightened to prevent further evaporation of water. Incubate tubes at room temperature for up to 14 days.

Expected Results

After 24-48 hours, most strains of *C. albicans* and *C. stellatoidea* will have formed typical chlamydospores.[3] Invert the plate and examine microscopically (100× magnification) for chlamydospore formation along the line of inoculation.

Growth of *T. rubrum* is pink to red on medium containing dextrose and, therefore, it is distinguishable from *T. mentagrophytes*.

Limitation of the Procedure

Polysorbate 80 enhances chlamydospore production by *C. albicans* and *C. stellatoidea*; however, it also enhances chlamydospore formation in other *Candida* species. Therefore, it is necessary to use additional media for species identification.[4]

References

1. Taschdjian. 1953. Mycologia *45*:474.
2. Taschdjian. 1957. Mycologia *49*:332.
3. Cooper and Silva-Hutner. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Rice Extract Agar

Cat. No. 211567 Dehydrated – 100 g

Rogosa SL Agar • Rogosa SL Broth

Intended Use
Rogosa SL Agar and Rogosa SL Broth are used for cultivating oral, vaginal and fecal lactobacilli.

Summary and Explanation
Rogosa SL Agar and Broth, also known as RMW Agar/Broth, are a modification of media described by Rogosa, Mitchell and Wiseman.[1,2] These media are used for isolation, enumeration and identification of lactobacilli in oral bacteriology, feces, vaginal specimens and foodstuffs.[3,4] The low pH and high acetate concentrations effectively suppress other bacterial flora allowing lactobacilli to flourish.

Principles of the Procedure
Peptone provides carbon and nitrogen. Yeast extract is a source of trace elements, vitamins and amino acids. Dextrose, arabinose and saccharose are carbohydrate sources that provide carbon. Sodium acetate and ammonium citrate inhibit streptococci, molds and other oral microbial flora and restrict swarming. Monopotassium phosphate provides buffering capability. Magnesium sulfate, manganese sulfate and ferrous sulfate are sources of inorganic ions. Polysorbate 80 acts as a surfactant. Agar is the solidifying agent.

Formulae
Difco™ Rogosa SL Agar
Approximate Formula* Per Liter

Tryptone	10.0	g
Yeast Extract	5.0	g
Dextrose	10.0	g
Arabinose	5.0	g
Saccharose	5.0	g
Sodium Acetate	15.0	g
Ammonium Citrate	2.0	g
Monopotassium Phosphate	6.0	g
Magnesium Sulfate	0.57	g
Manganese Sulfate	0.12	g
Ferrous Sulfate	0.03	g
Polysorbate 80	1.0	g
Agar	15.0	g

Difco™ Rogosa SL Broth
Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend the powder in 1 L of purified water:
   Difco™ Rogosa SL Agar – 75 g;
   Difco™ Rogosa SL Broth – 59.7 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 1.32 mL of glacial acetic acid and mix thoroughly.
4. Boil for 2-3 minutes. DO NOT AUTOCLAVE.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control
Identity Specifications
Difco™ Rogosa SL Agar

Dehydrated Appearance:	Beige, homogeneous with soft clumps.
Solution:	7.5% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent and may have a slight precipitate.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 7.5% Solution (with glacial acetic acid and reboiling) at 25°C:	pH 5.4 ± 0.2

Difco™ Rogosa SL Broth

Dehydrated Appearance:	Beige, appears moist, with soft clumps.
Solution:	6.0% solution, soluble in purified water upon boiling. Solution is light amber, clear to slightly opalescent.
Prepared Appearance:	Light amber, clear to slightly opalescent.
Reaction of 6.0% Solution (with glacial acetic acid and reboiling) at 25°C:	pH 5.4 ± 0.2

Cultural Response
Difco™ Rogosa SL Agar or Rogosa SL Broth
Prepare the medium per label directions. Inoculate the agar medium using the pour plate technique and incubate at 35 ± 2°C for 40-48 hours. Inoculate the broth medium and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus rhamnosus	9595	10^2-10^3	Good
Lactobacillus delbrueckii subsp. *lactis*	4797	10^2-10^3	Good
Staphylococcus aureus	25923	10^3-2×10^3	Marked to complete inhibition

Procedure
See appropriate references for specific procedures.

Expected Results
Refer to appropriate references and procedures for results.

Limitation of the Procedure
The salt in the formulation makes the media unsuitable for isolation of dairy lactobacilli; e.g., *L. lactis*, *L. bulgaricus* and *L. helveticus*.[4]

References
1. Rogosa, Mitchell and Wiseman. 1951. J. Bacteriol. *62*:132.
2. Rogosa, Mitchell and Wiseman. 1951. J. Dental Res. *30*:682.
3. Hall, Ledenbach and Flowers. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Rogosa SL Agar

COMPF

Cat. No. 248020 Dehydrated – 500 g*

Japan

Cat. No. 251542 Prepared Plates – Ctn. of 100*

Difco™ Rogosa SL Broth

COMPF

Cat. No. 247810 Dehydrated – 500 g*

**Store at 2-8°C.*

Rose Agar with 5% Sheep Blood

Intended Use

Rose Agar with 5% Sheep Blood is used in qualitative procedures for the isolation and cultivation of gram-positive cocci from clinical specimens.

Summary and Explanation

Rose Agar is a combination of the ingredients of two selective media formulations, Columbia CNA Agar base[1] and Phenylethyl Alcohol Agar base,[2] plus sheep blood. The medium was found to yield greater recovery of gram-positive cocci, particularly streptococci, than either medium individually. (Phenylethyl alcohol imparts a rose-like odor to the medium.)

Columbia CNA Agar is a selective and differential medium for gram-positive organisms; Phenylethyl Alcohol Agar is also a selective medium for the isolation of gram-positive bacteria, particularly gram-positive cocci.

Principles of the Procedure

Rose Agar is a nutritious medium containing amino acids, peptones and trace elements provided by enzymatic digests of casein, animal tissue, heart muscle and soybean meal. Yeast extract provides additional nutrients and vitamins. The inclusion of colistin and nalidixic acid renders the medium selective for gram-positive bacteria; phenylethyl alcohol is bacteriostatic for gram-negative organisms. The addition of 5% sheep blood to the medium allows for the detection of hemolytic reactions.[3]

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates, protected from light, in an inverted position (agar side up) at 35°C for 18-48 hours in an aerobic atmosphere supplemented with CO_2.

Expected Results

Streptococcal colonies will appear as small, white to grayish colonies, which may show beta or alpha hemolysis.

References

1. Ellner, Stoessel, Drakeford and Vasi. 1966. Am. J. Clin. Pathol. *45*:502.
2. Lilley and Brewer. 1953. J. Am. Pharm. Assoc. *42*:6.
3. Esterez. 1984. Lab. Med. *15*:258.

Availability

BBL™ Rose Agar with 5% Sheep Blood

Cat. No. 297961 Prepared Plates – Pkg. of 20*

297964 Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*

Rose Bengal Agar Base
Rose Bengal Antimicrobic Supplement C

Intended Use

Rose Bengal Agar Base is used with Rose Bengal Antimicrobic Supplement C in isolating and enumerating yeasts and molds.

Summary and Explanation

A number of methods have been described for the selective isolation of fungi from environmental materials and foodstuffs containing mixed populations of fungi and bacteria. The use of media with an acid pH that selectively inhibits the growth of bacteria and thereby promotes the growth of fungi has been widely employed.[1-3] A number of investigators have reported, however, that acidified media may actually inhibit fungal growth,[4,5] fail to completely inhibit bacterial growth[5] and have little effect in restricting the size of mold colonies.[6] Smith and Dawson[7] used rose bengal in a neutral pH medium for the selective isolation of fungi from soil samples. Chloramphenicol, streptomycin, oxytetracycline and chlortetracycline have been used for the improved, selective isolation and enumeration of yeasts and molds from soil, sewage and foodstuffs.[4,8-11]

Rose Bengal Agar Base supplemented with Rose Bengal Antimicrobic Supplement C is a modification of the Rose Bengal Chlortetracycline Agar formula of Jarvis.[11] Instead of chlortetracycline, chloramphenicol is employed in this medium as a selective supplement. Of the antibiotics most frequently employed in media of neutral pH, chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum. A modified formulation of Rose Bengal Agar is recommended in *Standard Methods for the Examination of Water and Wastewater* for the enumeration of yeasts and molds.[12]

User Quality Control

Identity Specifications

Difco™ Rose Bengal Agar Base

Dehydrated Appearance:	Beige to faint pink, free-flowing, homogeneous.
Solution:	3.2% solution, soluble in purified water upon boiling. Solution is reddish pink, very slightly to slightly opalescent.
Prepared Appearance:	Bright pink, very slightly to slightly opalescent.
Reaction of 3.2% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Rose Bengal Antimicrobic Supplement C

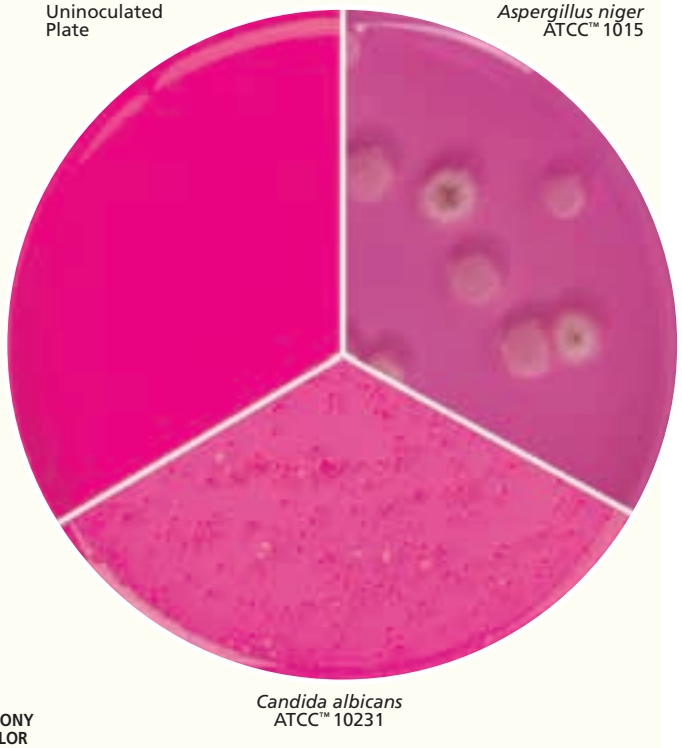
Lyophilized Appearance:	White cake, may be dispersed.
Rehydrated Appearance:	Colorless, clear.
Solubility:	Soluble in 3 mL ethanol.

Cultural Response

Difco™ Rose Bengal Agar Base with Antimicrobic Supplement C

Prepare the medium per label directions. Inoculate using the pour plate technique (for *Aspergillus niger*, inoculate the surface of an agar slant) and incubate aerobically at 25-30°C for up to 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Aspergillus niger	1015	Fresh	Good	White to black
Candida albicans	10231	10^2-3×10^2	Good	Pink
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition	–
Micrococcus luteus	10240	10^3-2×10^3	Marked to complete inhibition	–



The Hycheck™ hygiene contact slide is a double-sided paddle containing two agar surfaces for immersing into fluids or sampling surfaces. There are two slides with Rose Bengal Chloramphenicol Agar along with another medium: Tryptic Soy Agar; and Tryptic Soy Agar with 0.01% TTC.

Principles of the Procedure

Peptone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms. Dextrose is an energy source. Monopotassium phosphate provides buffering capability. Magnesium sulfate provides necessary trace elements. Rose bengal is included as a selective agent that inhibits bacterial growth and restricts the size and height of colonies of the more rapidly growing molds. The restriction in growth of molds aids in the isolation of slow-growing fungi by preventing overgrowth by more rapidly growing species. Rose bengal is taken up by yeast and mold colonies, thereby facilitating their recognition and enumeration. Rose Bengal Antimicrobic Supplement C is a lyophilized antimicrobic supplement containing chloramphenicol which inhibits bacteria. Agar is the solidifying agent.

Formulae

Difco™ Rose Bengal Agar Base

Approximate Formula* Per Liter

Soy Peptone	5.0	g
Dextrose	10.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Rose Bengal	0.05	g
Agar	15.0	g

Difco™ Rose Bengal Antimicrobic Supplement C

Approximate Formula* Per 3 mL Vial

Chloramphenicol	0.05	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Rose Bengal Agar Base

1. Suspend 16 g of the powder in 500 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 3 mL rehydrated Rose Bengal Antimicrobic Supplement C to 500 mL of cooled agar base (final concentration of chloramphenicol is 100 mg/L). Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Rose Bengal Antimicrobic Supplement C (Chloramphenicol)

1. Aseptically add 3 mL ethanol per vial of supplement.
2. Invert several times to dissolve the powder.

Procedure

1. Inoculate 0.1 mL of appropriate dilutions in duplicate on the solidified agar. Spread over the entire surface using a sterile bent glass rod.
2. Incubate plates at 25-30°C for up to 7 days.

Expected Results

Colonies of yeast appear pink due to the uptake of rose bengal. Count plates containing 15-150 colonies and report the counts as colony-forming units (CFU) per gram or mL of sample.

Limitations of the Procedure

1. Although this medium is selective primarily for fungi, microscopic examination is recommended for presumptive identification. Biochemical testing using pure cultures is required for complete identification.
2. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the complete medium; similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
3. Care should be taken not to expose this medium to light, since photodegradation of rose bengal yields compounds that are toxic to fungi.[13]

References

1. Waksman. 1922. J. Bacteriol. 7:339.
2. Koburger. 1976. *In* Speck (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
3. Mossel, Visser and Mengerink. 1962. Lab Practice *11*:109.
4. Martin. 1950. Soil Sci. *69*:215.
5. Koburger. 1972. J. Milk Food Technol. *35*:659.
6. Tyner. 1944. Soil Sci. *57*:271.
7. Smith and Dawson. 1944. Soil Sci. *58*:467.
8. Cooke. 1954. Antibiot. and Chemother. *4*:657.
9. Papavizas and Davey. 1959. Soil Sci. *88*:112.
10. Overcast and Weakley. 1969. J. Milk Technol. *32*:442.
11. Jarvis. 1973. J. Appl. Bacteriol. *36*:723.
12. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
13. Banks, Board and Paton. 1985. Lett. Appl. Microbiol. *1*:7.

Availability

Difco™ Rose Bengal Agar Base

SMWW

Cat. No. 218312 Dehydrated – 500 g

Difco™ Rose Bengal Antimicrobic Supplement C

SMWW

Cat. No. 214904 Vial – 10 × 3 mL*

Difco™ Hycheck™ Hygiene Contact Slides

Cat. No. 290006 Rose Bengal Chloramphenicol Agar// Tryptic Soy Agar – Pkg. of 10 slides*

290007 Rose Bengal Chloramphenicol Agar// Tryptic Soy Agar with 0.01% TTC – Pkg. of 10 slides*

**Store at 2-8°C.*

S

SF Medium • SF Broth

Intended Use

SF (Streptococcus Faecalis) Medium (Broth) is used for the differentiation of *Enterococcus* species from the *Streptococcus bovis* group and other streptococci.

Summary and Explanation

The formulation of SF Medium was developed by Hajna and Perry[1] as a result of their comparative study of presumptive and confirmatory media for the detection of coliforms and fecal streptococci. It was recommended for use in the examination of waters and other materials for the presence of fecal streptococci as an indicator of pollution. The use of SF Medium in sanitary bacteriology has been replaced by more selective media recommended in current compendia of methods for the examination of waters and foods.[2-4]

For diagnostic microbiology purposes, the medium is useful in differentiation of enterococci from streptococci. Pure cultures of streptococci are inoculated into SF Medium in order to determine if the respective culture is *Enterococcus* sp. Enterococci ferment dextrose and grow in the presence of the inhibitor sodium azide.

Principles of the Procedure

Peptone and dextrose supply the nutrients required for the growth of enterococci. Sodium chloride maintains the osmotic balance of the medium. Sodium azide exhibits a bacteriostatic effect on gram-negative bacteria through its inhibitory action on enzymes in the electron transport system. Bromcresol purple serves as a pH indicator.

Formula

Difco™ SF Medium

Approximate Formula* Per Liter		
Tryptone	20.0	g
Dextrose	5.0	g
Dipotassium Phosphate	4.0	g
Monopotassium Phosphate	1.5	g
Sodium Chloride	5.0	g
Sodium Azide	0.5	g
Bromcresol Purple	32.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ SF Medium

Dehydrated Appearance:	Light beige to gray, may have a light greenish tint, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in purified water. Solution is purple, clear.
Prepared Appearance:	Dark purple, clear to slightly hazy, may contain a slight precipitate.
Reaction of 3.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ SF Medium

Prepare the medium per label directions. Inoculate and incubate at 45-46°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Enterococcus faecalis	19433	10^5-10^6	Good	Yellow (acid)
Enterococcus faecium	27270	10^5-10^6	Good	Yellow (acid)
Escherichia coli	25922	10^5-10^6	Inhibition	No change
Streptococcus bovis	33317	10^5-10^6	None to poor	No change

Directions for Preparation from Dehydrated Product

1. Dissolve 36 g of the powder in 1 L of purified water. For double strength medium, use 72 g/L of purified water. Rehydrate with proportionally less water when liquid inocula will exceed 1 mL.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate tubes of the medium with pure cultures of the test organisms. Incubate tubes for 18-48 hours at 45-46°C in an aerobic atmosphere.

Expected Results

A positive reaction is indicated by turbidity and a yellow-brown color due to the fermentation of dextrose and the resultant color change of the bromcresol purple indicator.

A negative reaction is indicated by no change in the purple color of the medium.

Streptococci yielding positive reactions:

E. faecalis
E. faecium

Streptococci yielding negative reactions:

S. bovis
S. equinus
S. mitis
S. salivarius
Streptococcus species other than group D

Limitations of the Procedure

1. Pure cultures of enterococci (streptococci) should be inoculated into this medium.
2. Group D streptococci include both enterococcal and non-enterococcal strains. Consult appropriate references for further identification of group D streptococci.

References

1. Hajna and Perry. 1943. Am J. Public Health. *33*:550.
2. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
3. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
4. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ SF Medium

Cat. No. 231510 Dehydrated – 500 g

BBL™ SF Broth

Cat. No. 221712 Prepared Tubes – Ctn. of 100

SFP Agar Base
Egg Yolk Enrichment 50%
Antimicrobic Vial K • Antimicrobic Vial P

Intended Use

SFP Agar Base is used with Egg Yolk Enrichment 50%, Antimicrobic Vial P and Antimicrobic Vial K in detecting and enumerating *Clostridium perfringens* in foods.

Summary and Explanation

Shahidi Ferguson Perfringens (SFP) Agar Base is prepared according to the formulation of Shahidi and Ferguson.[1] With the addition of 50% egg yolk emulsion, both the lecithinase reaction and the sulfite reaction can identify *Clostridium perfringens*. The selectivity of the medium is due to the added kanamycin and polymyxin B.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients, but occurrences of foodborne illness are usually associated with cooked meat or poultry products.[2] Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.[3] Enumerating the microorganism

User Quality Control

Identity Specifications

Difco™ SFP Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	47 g, soluble in 900 mL purified water upon boiling. Solution is medium to dark amber, slightly opalescent.
Prepared Appearance (Final):	Canary yellow, opaque.
Reaction of 47g/900 mL Solution at 25°C:	pH 7.6 ± 0.2

Difco™ Egg Yolk Enrichment 50%

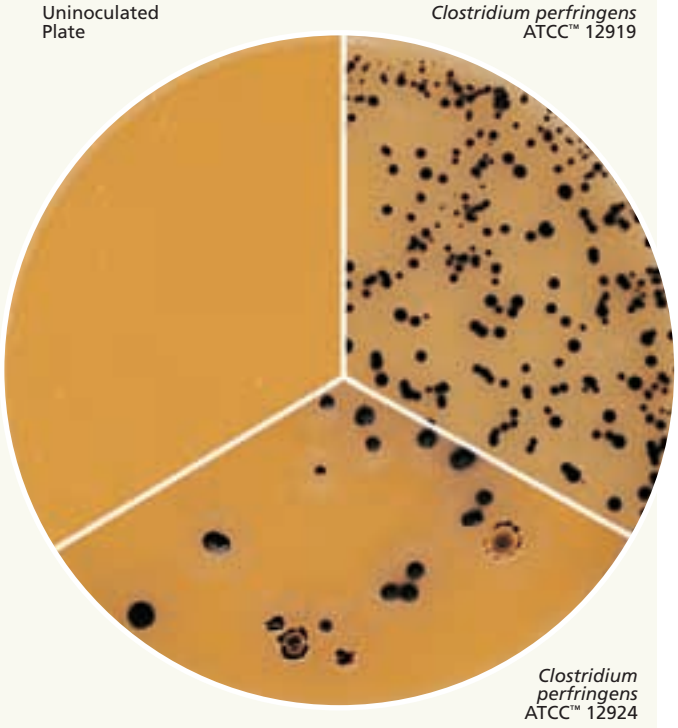
Appearance:	Canary yellow, opaque solution with a re-suspendable precipitate.

Difco™ Antimicrobic Vial K

Dehydrated Appearance:	White cake or powder.
Rehydrated Appearance:	Colorless, clear solution.

Difco™ Antimicrobic Vial P

Dehydrated Appearance:	White cake or powder.
Rehydrated Appearance:	Colorless, clear solution.



Cultural Response

Difco™ SFP Agar Base

Prepare the base layer and cover layer per label directions, inoculating the base layer. Incubate at 35 ± 2°C under anaerobic conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Clostridium perfringens	12919	30-300	Good	Black with a zone of precipitation (halo)
Clostridium perfringens	12924	30-300	Good	Black with a zone of precipitation (halo)

in food samples plays a role in the epidemiological investigation of outbreaks of foodborne illness.[2]

SFP Agar (with added kanamycin and polymyxin B) is comparable to Tryptose Sulfite Cycloserine (TSC) Agar, which uses cycloserine as the inhibitory component.[2,4,5]

Principles of the Procedure

SFP Agar Base contains peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins, which stimulate bacterial growth. Ferric ammonium citrate and sodium sulfite are H_2S indicators. Clostridia reduce sulfite to sulfide, which reacts with iron to form a black iron sulfide precipitate. Antimicrobic Vial P contains polymyxin B and Antimicrobic Vial K contains kanamycin; both are inhibitors to organisms other than *Clostridium* spp. Egg Yolk Enrichment 50% provides egg yolk lecithin, which some clostridia hydrolyze. Agar is the solidifying agent.

Formulae

Difco™ SFP Agar Base

Approximate Formula* Per Liter

Yeast Extract	5.0	g
Proteose Peptone No. 3	7.5	g
Pancreatic Digest of Casein	7.5	g
Soytone	5.0	g
Ferric Ammonium Citrate	1.0	g
Sodium Bisulfite	1.0	g
Agar	20.0	g

Difco™ Egg Yolk Enrichment 50%

Concentrated egg yolk emulsion.

Difco™ Antimicrobic Vial K

25 mg Kanamycin per 10 mL vial.

Difco™ Antimicrobic Vial P

30,000 units Polymyxin B per 10 mL vial.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ SFP Agar Base

Base Layer:

1. Suspend 47 g of the powder in 900 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50°C.
4. Add 100 mL Egg Yolk Enrichment 50%, 10 mL of rehydrated Antimicrobic Vial P (30,000 units polymyxin B sulfate) and 4.8 mL rehydrated Antimicrobic Vial K (12 mg kanamycin). Mix thoroughly.

Cover Layer:

1. Suspend 47 g of the powder in 1 L of purified water.
2. Prepare as above, except omit Egg Yolk Enrichment 50%.
3. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Antimicrobic Vial K (Kanamycin)

1. To rehydrate, aseptically add 10 mL sterile purified water per vial.
2. Rotate in an end-over-end motion to dissolve the contents completely.

Difco™ Antimicrobic Vial P (Polymyxin B)

1. To rehydrate, aseptically add 10 mL of sterile purified water per vial.
2. Rotate in an end-over-end motion to dissolve the contents completely.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Shahidi and Ferguson. 1971. Appl. Microbiol. *21*:500.
2. Labbe. 2001. *In* Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ SFP Agar Base

AOAC BAM COMPF ISO

Cat. No. 281110 Dehydrated – 500 g

Difco™ Antimicrobic Vial K

Cat. No. 233391 Vial – 6 × 10 mL*

Difco™ Antimicrobic Vial P

Cat. No. 232681 Vial – 6 × 10 mL*

Difco™ Egg Yolk Enrichment 50%

AOAC BAM COMPF

Cat. No. 233471 Bottle – 12 × 10 mL*
233472 Bottle – 6 × 100 mL*

**Store at 2-8°C.*

SIM Medium

Intended Use

SIM Medium is used to differentiate enteric bacilli on the basis of sulfide production, indole formation and motility.

Summary and Explanation

Hydrogen sulfide production, indole formation and motility are distinguishing characteristics which aid in the identification of the *Enterobacteriaceae*, especially *Salmonella* and *Shigella*. SIM Medium, therefore, is useful in the process of identification of enteric pathogens.

Principles of the Procedure

The ingredients in SIM Medium enable the determination of three activities by which enteric bacteria can be differentiated. Sodium thiosulfate and ferrous ammonium sulfate are indicators of hydrogen sulfide production. The ferrous ammonium sulfate reacts with H_2S gas to produce ferrous sulfide, a black precipitate.[1] The casein peptone is rich in tryptophan, which is attacked by certain microorganisms resulting in the production of indole. The indole is detected by the addition of chemical reagents following the incubation period. Motility detection

User Quality Control

Identity Specifications

BBL™ SIM Medium

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ SIM Medium

Prepare the medium per label directions. Stab inoculate using heavy inocula of fresh cultures and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	RECOVERY	MOTILITY	H_2S	INDOLE
Escherichia coli	25922	Good	+	–	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	13311	Good	+	+	–
Shigella sonnei	9290	Good	–	–	–

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 with indole reagent | *Salmonella* Typhimurium ATCC™ 13311 with indole reagent

is possible due to the semisolid nature of the medium. Growth radiating out from the central stab line indicates that the test organism is motile.

Formula

BBL™ SIM Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	20.0	g
Peptic Digest of Animal Tissue	6.1	g
Ferrous Ammonium Sulfate	0.2	g
Sodium Thiosulfate	0.2	g
Agar	3.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 30 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Loosen caps, boil and cool before use. Using growth from a pure culture, stab an inoculating needle two-thirds of the distance to the bottom in the center of the tube. Incubate tubes with loosened caps for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Following incubation, observe for motility (diffuse growth outward from the stab line or turbidity throughout the medium) and for H_2S production (blackening along the stab line). To detect indole production, add three or four drops of Kovacs' reagent[2] and observe for a red color (positive reaction).

Consult appropriate references for activities of specific microorganisms.[2-4]

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
3. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
4. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ SIM Medium

BAM

Cat. No.		
	211578	Dehydrated – 500 g
	221010	Prepared Tubes – Pkg. of 10
	221011	Prepared Tubes – Ctn. of 100

S

SOB Medium

Intended Use

SOB Medium is used for cultivating recombinant strains of *Escherichia coli*.

User Quality Control

Identity Specifications

Difco™ SOB Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.8% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 2.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ SOB Medium

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli (DH-5)	53868	10^2-3×10^2	Good

Summary and Explanation

SOB Medium was developed by Hanahan[1] as a nutritionally rich growth medium for preparation and transformation of competent cells. Transformation requires making perforations in the bacterium (i.e., making the cells "competent") to allow the introduction of foreign DNA into the cell. To survive this process, competent cells need a rich, isotonic environment.

SOC Medium, used in the final stage of transformation, may be prepared by aseptically adding 20 mL of a filter-sterilized 20% solution of glucose (dextrose) to the sterile SOB Medium. This addition provides a readily available source of carbon and energy in a form *E. coli* can use in mending the perforations and for replication.[2]

Principles of the Procedure

Peptone and yeast extract provide sources of nitrogen and growth factors which allow the bacteria to recover from the stress of transformation and grow well. Sodium chloride and potassium chloride provide essential ions. Magnesium sulfate is a source of magnesium ions required in a variety of enzymatic reactions, including DNA replication.

Formula

Difco™ SOB Medium

Approximate Formula* Per Liter		
Tryptone	20.0	g
Yeast Extract	5.0	g
Sodium Chloride	0.5	g
Magnesium Sulfate (anhydrous)	2.4	g
Potassium Chloride	186.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 28 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. If desired, SOC Medium may be prepared by adding 20 mL filter-sterilized 20% glucose solution after cooling the medium to 45-50°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[2]

Expected Results

Growth is evident in the form of turbidity.

References

1. Hanahan. 1983. J. Mol. Biol. *166*:557.
2. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Availability

Difco™ SOB Medium

Cat. No. 244310 Dehydrated – 500 g

SPS Agar

Intended Use

SPS Agar is used for detecting and enumerating *Clostridium perfringens* in food.

Summary and Explanation

In the 1950s, Mossel[1] and Mossel et al.[2] proposed media for enumerating anaerobic sulfite-reducing clostridia in foods. Angelotti et al.[3] modified the formula as Sulfite Polymyxin Sulfadiazine (SPS) Agar and used it to quantitate *C. perfringens* in foods.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients. Occurrences of foodborne illness from *C. perfringens* are usually associated with cooked meat or poultry products.[4] Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.[5] Enumerating the microorganism in food samples plays a role in epidemiological investigation of outbreaks of foodborne illness.[4]

Principles of the Procedure

SPS Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Ferric citrate and sodium sulfite are H_2S indicators. Clostridia reduce the sulfite to sulfide, which reacts with the iron from ferric citrate to form a black iron sulfide precipitate. Polysorbate 80 is a dispersing agent. Polymyxin B sulfate and sulfadiazine are inhibitors to organisms other than *Clostridium* spp. Sodium thioglycollate is a reducing agent. Agar is the solidifying agent.

Formula

Difco™ SPS Agar

Approximate Formula* Per Liter		
Tryptone	15.0	g
Yeast Extract	10.0	g
Ferric Citrate	0.5	g
Sodium Sulfite	0.5	g
Sodium Thioglycollate	0.1	g
Polysorbate 80	0.05	g
Sulfadiazine	0.12	g
Polymyxin B Sulfate	0.01	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 41 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Dispense inoculum into sterile Petri dish.
2. Pour medium cooled to 50-55°C over the inoculum.
3. Gently but thoroughly mix the inoculum and medium. Allow to solidify on a flat surface.
4. Incubate anaerobically at 35 ± 2°C for 24-48 hours.

Expected Results

Clostridium perfringens will grow as black colonies with good growth.

User Quality Control

Identity Specifications

Difco™ SPS Agar

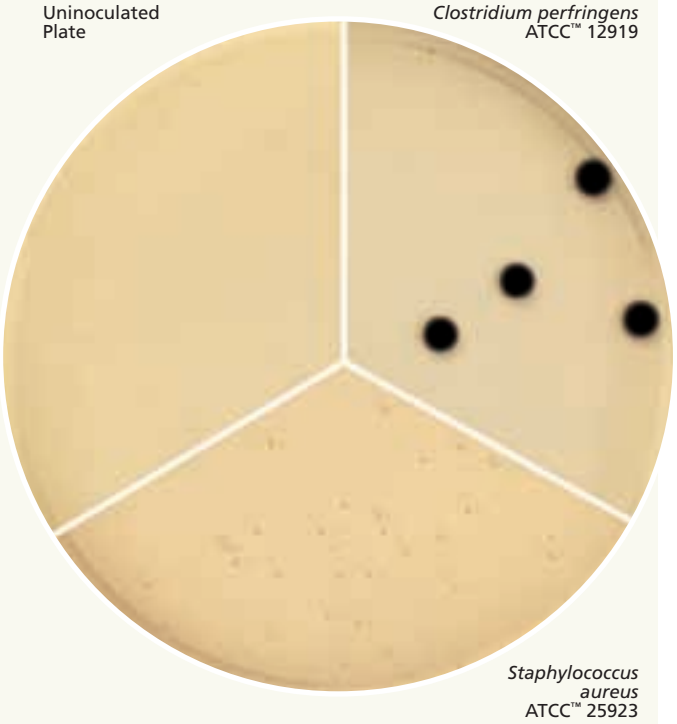
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ SPS Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate anaerobically at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Clostridium perfringens	12919	10^2-10^3	Good	Black
Clostridium sporogenes	11437	10^2-10^3	None to fair	Black
Escherichia coli	25922	10^2-10^3	Marked to complete inhibition	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Marked to complete inhibition	–
Staphylococcus aureus	25923	10^2-10^3	Fair to good	White



Limitation of the Procedure

The high degree of selectivity of SPS Agar may inhibit some strains of *C. perfringens* while other strains that grow may fail to produce distinguishing black colonies.[4]

Availability

Difco™ SPS Agar

Cat. No. 284530 Dehydrated – 500 g*

**Store at 2-8°C.*

References

1. Mossel. 1959. J. Sci. Food Agric. *19*:662.
2. Mossel, DeBruin, van Diepen, Vendrig and Zoutewelle. 1956. J. Appl. Microbiol. *19*:142.
3. Angelotti, Hall, Foster and Lewis. 1962. Appl. Microbiol. *10*:193.
4. Labbe. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

SS Agar • Salmonella Shigella Agar

Intended Use

SS Agar and Salmonella Shigella Agar are moderately selective and differential media for the isolation of pathogenic enteric bacilli, especially those belonging to the genus *Salmonella*. This formulation is not recommended for the primary isolation of *Shigella*.

Summary and Explanation

The culture media that have been developed for the selection and differentiation of enteric microorganisms from clinical and nonclinical materials inhibit the growth of gram-positive species to a varying degree due to the presence of either pure bile salts, mixtures of bile salts or dyes. SS Agar and Salmonella Shigella Agar are examples of media used in the plating of samples for the detection of enteric pathogens that contain bile salt mixtures. This formulation is essentially a modification of the Desoxycholate-Citrate Agar described by Leifson.[1]

Principles of the Procedure

SS Agar and Salmonella Shigella Agar are designated as moderately selective media based upon the degree of inhibition of gram-positive microorganisms that they inhibit due to their content of bile salts, brilliant green and citrates. Differentiation of enteric organisms is achieved by the incorporation of lactose in the medium. Organisms that ferment lactose produce acid which, in the presence of the neutral red indicator, results in the formation of red colonies. Lactose nonfermenters form colorless colonies. The latter group contains the majority of the intestinal pathogens, including *Salmonella* and *Shigella*.

The sodium thiosulfate and ferric citrate enable the detection of hydrogen sulfide production as evidenced by colonies with black centers.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ SS Agar

Dehydrated Appearance:	Very light buff to pink, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in purified water upon boiling. Solution is red-orange, very slightly to slightly opalescent.
Prepared Appearance:	Red-orange, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ SS Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	H_2S
Enterococcus faecalis	29212	10^3-2×10^3	Partial inhibition	Colorless	–
Escherichia coli	25922	10^3-2×10^3	Partial inhibition	Pink to red	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Colorless	+
Shigella flexneri	12022	10^2-10^3	Fair to good	Colorless	–

Identity Specifications

BBL™ Salmonella Shigella Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain many small tan flecks.
Solution:	6.0% solution, soluble in purified water upon boiling. Solution is medium, tan-orange to tan-red, clear to moderately hazy.
Prepared Appearance:	Medium, tan-orange to tan-red, clear to moderately hazy.
Reaction of 6.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

BBL™ Salmonella Shigella Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	H_2S
Enterococcus faecalis	29212	10^4-10^5	Complete inhibition	–	–
Escherichia coli	25922	10^4-10^5	Partial to complete inhibition	Pink to red	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	+
Shigella flexneri	12022	10^3-10^4	Good	Colorless	–

Formulae

Difco™ SS Agar

Approximate Formula* Per Liter

Beef Extract	5.0	g
Proteose Peptone	5.0	g
Lactose	10.0	g
Bile Salts No. 3	8.5	g
Sodium Citrate	8.5	g
Sodium Thiosulfate	8.5	g
Ferric Citrate	1.0	g
Agar	13.5	g
Brilliant Green	0.33	mg
Neutral Red	25.0	mg

BBL™ Salmonella Shigella Agar

Approximate Formula* Per Liter

Beef Extract	5.0	g
Pancreatic Digest of Casein	2.5	g
Peptic Digest of Animal Tissue	2.5	g
Lactose	10.0	g
Bile Salts	8.5	g
Sodium Citrate	8.5	g
Sodium Thiosulfate	8.5	g
Ferric Citrate	1.0	g
Agar	13.5	g
Brilliant Green	0.33	mg
Neutral Red	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 60 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool the medium to approximately 45-50°C and pour into Petri dishes.
4. Allow the plates to dry for approximately 2 hours with the covers partially removed.
5. Test samples of the finished product for performance using stable, typical control cultures.

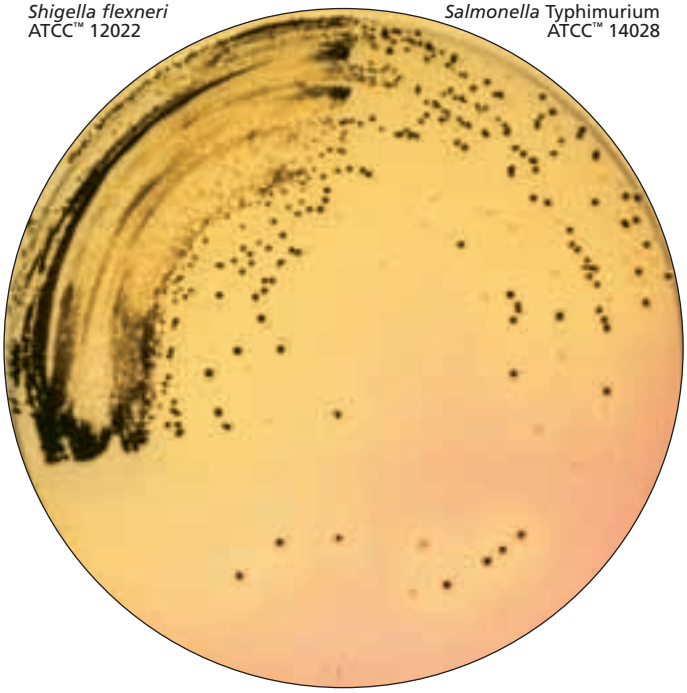
Procedure

Use standard procedures to obtain isolated colonies from specimens. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Expected Results

Typical colonial morphology on Salmonella Shigella Agar is as follows:

Escherichia coli	Slight growth, pink or red
Enterobacter/Klebsiella	Slight growth, pink
Proteus	Colorless, usually with black center
Salmonella	Colorless, usually with black center
Shigella	Colorless
Pseudomonas	Irregular, slight growth
Gram-positive bacteria	No growth



Limitation of the Procedure

Due to the relatively high level of selectivity, some *Shigella* strains may not grow on SS Agar and Salmonella Shigella Agar and, therefore, these media are not recommended for the primary isolation of *Shigella*.[1,2] Media recommended for the isolation of *Shigella* are Hektoen Enteric and XLD agars.[3]

References

1. Leifson. 1935. J. Pathol. Bacteriol. *40*:581.
2. Taylor and Harris. 1965. Am. J. Clin. Pathol. *44*:476.
3. Pollock and Dahlgren. 1974. Appl. Microbiol. *27*:197.

Availability

Difco™ SS Agar

BS12 CMPH2 COMPF MCM9

Cat. No.	274500	Dehydrated – 500 g
	212118	Dehydrated – 2 kg
	274300	Dehydrated – 10 kg

BBL™ Salmonella Shigella Agar

BS12 CMPH2 COMPF MCM9

Cat. No.	211596	Dehydrated – 100 g
	211597	Dehydrated – 500 g
	211600	Dehydrated – 5 lb (2.3 kg)
	293306	Dehydrated – 25 lb (11.3 kg)

United States and Canada

Cat. No.	221181	Prepared Plates – Pkg. of 20*
	221279	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254047	Prepared Plates – Pkg. of 20*
	254085	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251181	Prepared Plates – Pkg. of 20*
	251279	Prepared Plates – Ctn. of 100*
	251134	Prepared Plates – Ctn. of 200*
	251826	Prepared **I Plate**™ Dishes – Ctn. of 200*

BBL™ Salmonella Shigella Agar//Hektoen Enteric Agar

Cat. No.	297426	Prepared **I Plate**™ Dishes – Pkg. of 20*

**Store at 2-8°C.*

S

SXT Blood Agar

Intended Use

SXT Blood Agar is used in the isolation of Lancefield groups A and B streptococci from throat cultures and other specimens. The growth of viridans streptococci, other beta-hemolytic and nonhemolytic streptococci, most *Enterobacteriaceae*, *Neisseria* species and some *Pseudomonas* species is inhibited.

Summary and Explanation

Groups A and B streptococcal infections may cause serious medical complications. Group A streptococcal infections may result in scarlet fever, rheumatic fever or acute glomerulonephritis. Group B infections may produce neonatal sepsis and meningitis.[1]

To aid the detection of group A and B streptococci, chemicals (such as crystal violet and sodium azide) and antimicrobial agents (such as neomycin and gentamicin) have been incorporated into sheep blood agar.[2-4] These inhibitory agents suppress the growth of normal flora and other organisms that could mask the presence of group A and B streptococci.

Gunn et al. introduced SXT Sheep Blood Agar, consisting of **Trypticase**™ Soy Agar with 5% Sheep Blood and two antimicrobial agents, sulfamethoxazole and trimethoprim, for the isolation of group A and B streptococci from throat cultures.[5] They reported that most normal flora and beta-hemolytic streptococci other than groups A and B were inhibited on the SXT agar, resulting in the recovery of 42% more group A and 49% more group B streptococci than with sheep blood agar alone.

In similar studies, Mirrett and Reller and others have reported that SXT agar is more sensitive and more specific in the recovery of group A streptococci than sheep blood agar alone.[6-9]

Principles of the Procedure

SXT Blood Agar is a primary plating medium suitable for isolating group A streptococci (*S. pyogenes*) and group B streptococci (*S. agalactiae*) from clinical specimens. Sulfamethoxazole and trimethoprim act synergistically in this medium to suppress the growth of normal flora.[5] Defibrinated sheep blood supplies the nutrients necessary to support the growth of streptococci and, simultaneously, it allows detection of hemolytic reactions. Sheep blood also inhibits the growth of *Haemophilus haemolyticus*, a bacterium commonly found in nose and throat cultures that is indistinguishable from beta-hemolytic streptococci.[10]

Procedure

Streak the specimen as soon as possible after it is received in the laboratory. For swab specimens, inoculate the medium by rolling the swab over a third of the agar surface and streaking the remainder of the plate to obtain isolated colonies. Stab the medium several times with the inoculating loop in the area of the heaviest inoculation to enhance detection of beta hemolysis. Material not being cultured from swabs may be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Incubate the plates in an inverted position (agar side up) for 18-24 hours at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide. Negative plates should be reincubated for an additional 18-24 hours.[11]

Expected Results

After 18-48 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Group A or B streptococci may be presumptively identified as small, translucent to opaque, white to gray colonies surrounded by zones of beta hemolysis. Gram stains, biochemical tests, susceptibility to bacitracin, utilizing **Taxo**™ A (0.04 unit) discs, and serological procedures should be performed to confirm findings.

Pinpoint colonies of alpha-hemolytic, nonhemolytic or other beta-hemolytic streptococci may grow in small numbers on the medium, but should not interfere with the recovery of group A and B streptococci or the interpretation of the results. *Neisseria*, coliforms, viridans streptococci and most non-group A or B streptococci are inhibited. Some staphylococci and *Pseudomonas* species may not be inhibited.

References

1. Spellerberg and Brandt. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Facklam. 1976. CRC Crit. Rev. Clin. Lab. Sci. *6*:287.
3. Blanchette and Lawrence. 1967. Am. J. Clin. Pathol. *48*:441.
4. Black and Van Buskirk. 1973. J. Clin. Pathol. *26*:154.
5. Gunn, Onashi, Gaydos and Holt. 1977. J. Clin. Microbiol. *5*:650.
6. Mirrett and Reller. 1984. Abstr. C203, p. 270. Abstr. 84th Annu. Meet. Am. Soc. Microbiol. 1984.
7. Kurzynski and Meise. 1979. J. Clin. Microbiol. *9*:189.
8. Kurzynski and Van Holten. 1981. J. Clin. Microbiol. *13*:891.
9. Kurzynski, Meise, Daggs and Helstad. 1979. J. Clin. Microbiol. *9*:144.
10. Ruoff. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
11. Kellog. 1990. J. Clin. Microbiol. *28*:165.

Availability

BBL™ SXT Blood Agar

BS12 CMPH2 MCM9

Cat. No.	221809	Prepared Plates – Pkg. of 20*
	221810	Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*

Sabouraud Brain Heart Infusion Agar Base Sabouraud Brain Heart Infusion Agar • Sabouraud Brain Heart Infusion Agar with Antimicrobics

Intended Use

Sabouraud Brain Heart Infusion Agar is used in qualitative procedures for cultivation of dermatophytes and other pathogenic and nonpathogenic fungi from clinical and nonclinical specimens. The medium is rendered selective by the addition of antimicrobial agents.

Summary and Explanation

Sabouraud Brain Heart Infusion Agar is based on the formulation of Gorman.[1] The combination of Brain Heart Infusion Agar and Sabouraud Dextrose Agar in this medium improves the recovery of fungi compared with the recovery on either medium individually. The addition of defibrinated sheep blood is recommended to increase the recovery of fastidious, dimorphic fungi.[2]

The antimicrobial agents chloramphenicol, cycloheximide and gentamicin are incorporated in various combinations to improve the recovery of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.[2]

Principles of the Procedure

Peptones and brain heart digest are sources of amino acids, nitrogen, sulfur, carbon and trace ingredients. Dextrose is an energy source for the metabolism of microorganisms. Sodium chloride provides essential electrolytes. Disodium phosphate buffers the medium to maintain the pH. Defibrinated sheep blood is added to supply nutrients that induce the growth of dimorphic species in the yeast phase.[2]

Chloramphenicol is a broad-spectrum antibiotic that inhibits a wide range of gram-negative and gram-positive bacteria. Cycloheximide is an antifungal agent that is primarily active against saprophytic fungi and does not inhibit pathogenic species. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria.

User Quality Control

Identity Specifications

Difco™ Sabouraud Brain Heart Infusion Agar Base

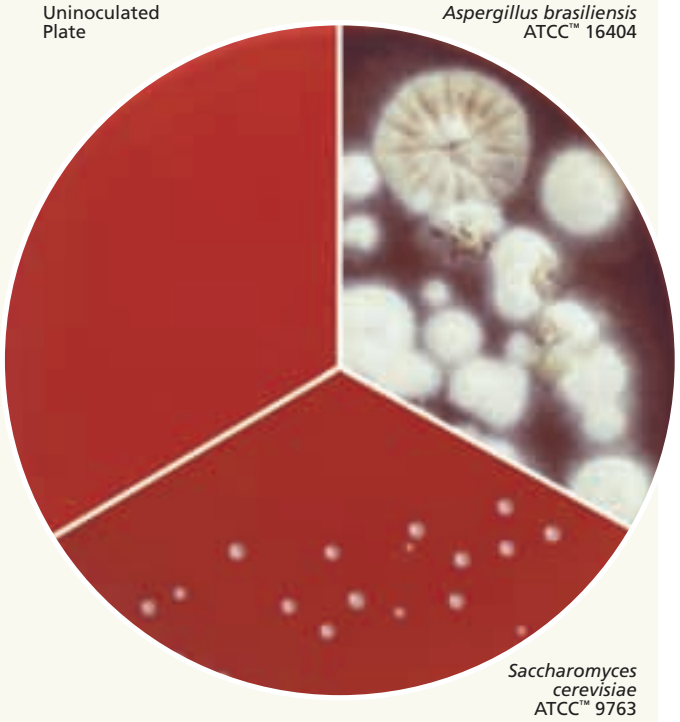
Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.9% solution, soluble in purified water upon boiling. Solution is medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Medium amber, very slightly to slightly opalescent.
Reaction of 5.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Sabouraud Brain Heart Infusion Agar Base

Prepare the medium per label directions without (plain) and with 10% sheep blood (SB). Inoculate and incubate at 30 ± 2°C for 18-48 hours and up to 7 days for *Trichophyton*.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN AND WITH SB
Aspergillus brasiliensis (niger)	16404	10^2-10^3	Good
Candida albicans	10231	10^2-10^3	Good
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition
Saccharomyces cerevisiae	9763	10^2-10^3	Good
Staphylococcus aureus	25923	10^3-2×10^3	Marked to complete inhibition
Trichophyton mentagrophytes	9533	10^2-10^3	Good



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Formula

Difco™ Sabouraud Brain Heart Infusion Agar Base

Approximate Formula* Per Liter

Brain Heart Digest	9.25	g
Proteose Peptone	5.0	g
Enzymatic Digest of Casein	5.0	g
Dextrose	21.0	g
Sodium Chloride	2.5	g
Disodium Phosphate	1.25	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 59 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Aseptically add 1 mL chloramphenicol solution (100 mg/mL) and, if desired, 10% sterile sheep blood.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens.

For isolation of fungi from potentially contaminated specimens, both a nonselective and a selective medium should be inoculated. Incubate the plates at 25-30°C in an inverted position (agar side up) with increased humidity. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C.

All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Expected Results

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Examine the plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Some fungi may be inhibited by the antibiotics in selective formulations.[3,4]

References

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Availability

Difco™ Sabouraud Brain Heart Infusion Agar Base

Cat. No. 279720 Dehydrated – 500 g

BBL™ Sabouraud Brain Heart Infusion Agar

BS12 MCM9

Cat. No. 297802 Prepared Plates (Deep Fill) – Pkg. of 10*
298192 Prepared Plates (Deep Fill) – Ctn. of 100*
297691 Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide

Cat. No. 297803 Prepared Plates (Deep Fill) – Pkg. of 10*
297692 Prepared Slants – Ctn. of 100*

BBL™ Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Gentamicin

MCM9

Cat. No. 297252 Prepared Slants – Pkg. of 10*

BBL™ Sabouraud Brain Heart Infusion Sheep Blood Agar with Chloramphenicol

Cat. No. 296307 **Mycoflask**™ Bottles – Pkg. of 10*

**Store at 2-8°C.*

Sabouraud Media (Low pH)
Sabouraud Dextrose Agar • Sabouraud Dextrose Agar with Antimicrobics • Sabouraud Dextrose Agar with Lecithin and Polysorbate 80 • Sabouraud Dextrose Broth • Sabouraud Maltose Agar Sabouraud Maltose Broth • Fluid Sabouraud Medium

Intended Use

Sabouraud Dextrose Agar is used in qualitative procedures for cultivation of pathogenic and nonpathogenic fungi, particularly dermatophytes. The medium is rendered more selective for fungi by the addition of antimicrobics. Sabouraud Dextrose Broth and Sabouraud Maltose Agar and Broth are also used for culturing yeasts, molds and aciduric microorganisms.

Fluid Sabouraud Medium is used for cultivating yeasts, molds and aciduric microorganisms and for detecting yeasts and molds in normally sterile materials.

Sterile Pack **RODAC**™ environmental sampling plates, containing Sabouraud Dextrose Agar with Lecithin and Polysorbate 80, are used for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Sterile Pack plates are particularly useful for monitoring surfaces in clean rooms and other environmentally-controlled areas and are also recommended for use in air sampling equipment, such as the Surface Air System.

Sterile Pack **Finger Dab**™ Isolator plates are intended for sampling gloved hands.

Sabouraud Dextrose Agar and Sabouraud Dextrose Broth meet *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Sabouraud Dextrose Agar is a general-purpose medium devised by Sabouraud for the cultivation of dermatophytes.[4] The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes, and slightly inhibitory to contaminating bacteria in clinical specimens.[5-8] Sabouraud Dextrose Agar is also recommended for the testing of cosmetics[9] and food.[10,11] General Chapters <61> and <62> of the *USP* describe test methods for using Sabouraud Dextrose Agar when performing the microbial enumeration tests and tests for isolating *Candida albicans* from nonsterile pharmaceutical products.[1]

The addition of antimicrobics is a modification designed to increase bacterial inhibition.

Sabouraud Dextrose Agar is also available in **RODAC**™ (Replicate Organism Detection and Counting) environmental sampling plates. These plates are specially constructed so that an agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden. **RODAC** plates are used in a variety of programs to establish and monitor cleaning techniques and schedules.[12-16] After touching the surface to be sampled with the medium, the environmental sampling dish is covered and incubated at an appropriate temperature. The presence and number of microorganisms is determined by the appearance of colonies on the surface of the agar medium. Collection of samples from the same area before and after cleaning and treatment with a disinfectant permits the evaluation of the efficacy of sanitary procedures. The **RODAC** SL (secure lid) has three lugs on the base, providing a tight fit between lid and base to reduce accidental contamination.

Sabouraud Maltose Agar is a modification of Sabouraud Dextrose Agar with maltose substituted for the dextrose. It is a selective medium due to the acid pH. Davidson et al. reported that Sabouraud Maltose Agar was a satisfactory medium in their studies of infections caused by *Microsporum audouini,*

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Sabouraud Dextrose Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 5.6 ± 0.2

Difco™ Sabouraud Dextrose Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 3.0% Solution at 25°C:	pH 5.6 ± 0.2

Difco™ Fluid Sabouraud Medium

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 3.0% Solution at 25°C:	pH 5.7 ± 0.2

Difco™ Sabouraud Maltose Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Very light amber, slightly opalescent without significant precipitate.
Reaction of 6.5% Solution at 25°C:	pH 5.6 ± 0.2

Difco™ Sabouraud Maltose Broth

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in purified water. Solution is light amber, clear to slightly opalescent.
Prepared Appearance:	Light amber, clear to slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Difco™ Sabouraud Dextrose Agar

Prepare the medium per label directions. Inoculate and incubate at 30± 2°C for 18-48 hours, or up to 7 days for *Trichophyton*. Incubate (*) cultures at 20-25°C for up to 5 days. Incubate (**) culture at 30-35°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Saccharomyces cerevisiae	9763	100-300	Good
Trichophyton mentagrophytes	9533	Undiluted	Good
*Aspergillus brasiliensis (niger)**	16404	<100	Growth
*Candida albicans**	10231	<100	Growth
*Candida albicans***	10231	<100	Growth

Difco™ Sabouraud Dextrose Broth

Prepare the medium per label directions. Inoculate tubes and incubate at 30± 2°C for 18-48 hours or up to 7 days if necessary. For (*) culture inoculate a 125 mL bottle and incubate at 30-35°C for 48 hours. For (**) culture inoculate a 125 mL bottle and incubate at 20-25°C for 3 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	10231	30-300	Good
Lactobacillus casei	9595	30-300	Good
Saccharomyces cerevisiae	9763	30-300	Good
*Candida albicans**	10231	<100	Growth
*Candida albicans***	10231	<100	Growth

Difco™ Fluid Sabouraud Medium

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	<100	Good
Candida albicans	10231	<100	Good
Saccharomyces cerevisiae	9763	<100	Good

Difco™ Sabouraud Maltose Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	10231	30-300	Good
Saccharomyces cerevisiae	9763	30-300	Good
Trichophyton mentagrophytes	9533	30-300	Good

Difco™ Sabouraud Maltose Broth

Prepare the medium per label directions. Inoculate tubes and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	10231	30-300	Good
Escherichia coli	25922	30-300	Good
Lactobacillus casei	9595	30-300	Good
Saccharomyces cerevisiae	9763	30-300	Good

Continued

Identity Specifications

BBL™ Sabouraud Dextrose Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain a large number of minute to small tan specks.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is pale to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Pale to medium, yellow to tan, clear to slightly hazy.
Reaction of 6.5% Solution at 25°C:	pH 5.6 ± 0.2

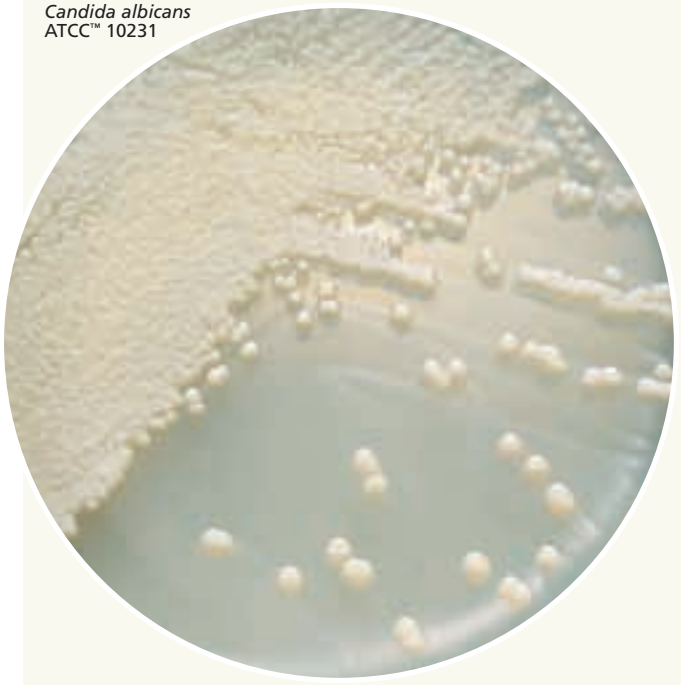
BBL™ Sabouraud Dextrose Agar (prepared)

Appearance:	Light to medium tan cream to yellow; trace hazy.
Reaction at 25°C:	pH 5.6 ± 0.2

BBL™ Sabouraud Dextrose Broth (prepared)

Appearance:	Light to medium tan yellow; clear to trace hazy.
Reaction at 25°C:	pH 5.6 ± 0.2

Candida albicans
ATCC™ 10231



Cultural Response

BBL™ Sabouraud Dextrose Agar

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for 7 days. Incubate (*) cultures at 20-25°C for up to 5 days. Incubate (**) culture at 30-35°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aureobasidium pullulans	9348	Undiluted	Good
Blastomyces dermatitidis	56218	Undiluted	Good
Candida albicans	60193	Undiluted	Good
Cryptococcus neoformans	32045	Undiluted	Good
Microsporum audouinii	9079	Undiluted	Good
Nocardia asteroides	19247	Undiluted	Good
Penicillium roquefortii	9295	Undiluted	Good
Trichophyton mentagrophytes	9533	Undiluted	Good
*Aspergillus brasiliensis (niger)**	16404	<100	Growth
*Candida albicans**	10231	<100	Growth
*Candida albicans***	10231	<100	Growth

BBL™ Sabouraud Dextrose Agar (prepared)

Inoculate and incubate at 25 ± 2°C for 7 days. Incubate *Candida albicans* ATCC™ 60193 and *Trichophyton* for 3 days. Incubate (*) cultures at 20-25°C; 5 days for *Aspergillus* and 2 days for *Candida*. Incubate (**) culture at 30-35°C for 2 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Candida albicans	60193	Undiluted	Good
Microsporum audouinii	9079	Undiluted	Good
Nocardia asteroides	19247	Undiluted	Good
Penicillium roquefortii	9295	Undiluted	Good
Trichophyton mentagrophytes	9533	Undiluted	Good
*Aspergillus brasiliensis (niger)**	16404	<100	Growth
*Candida albicans**	10231	<100	Growth
*Candida albicans***	10231	<100	Growth

BBL™ Sabouraud Dextrose Broth (prepared)

Inoculate and incubate *Aspergillus* at 20-25°C for 5 days. Inoculate *Candida albicans* in duplicate and incubate (*) culture at 20-25°C and (**) culture at 30-35°C for up to 5 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Growth
*Candida albicans**	10231	10-100	Growth
*Candida albicans***	10231	10-100	Growth

M. lanosum and *Trichophyton gypseum*.[17] Davidson and Dowding also used this medium in isolating *T. gypseum* from a case of tinea barbae.[18]

Sabouraud Dextrose Broth is used for culturing yeasts and molds in cosmetics.[9] General Chapter <62> of the *USP* recommends the use of Sabouraud Dextrose Broth when isolating *Candida albicans* from nonsterile pharmaceutical products.[1]

Sabouraud Maltose Broth is a modification of Sabouraud Dextrose Broth in which maltose is substituted for dextrose. It is selective due to its acid pH and is used for the detection of fungi.

Fluid Sabouraud Medium is employed in sterility test procedures for determining the presence of molds, yeasts and aciduric microorganisms. The acid reaction of the final medium is inhibitive to a large number of bacteria and makes the medium particularly well suited for cultivating fungi and acidophilic microorganisms.

Principles of the Procedure

Sabouraud dextrose media are peptone media supplemented with dextrose to support the growth of fungi. Sabouraud agar is also available with maltose substituted for the dextrose. Peptones are sources of nitrogenous growth factors. The carbohydrate provides an energy source for the growth of microorganisms. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria. Chloramphenicol is inhibitory to a wide range of gram-negative and gram-positive bacteria, and cycloheximide is an antifungal agent that is primarily active against saprophytic fungi and does not inhibit yeasts or dermatophytes.[19]

Lecithin neutralizes quaternary ammonium compounds, and polysorbate 80 neutralizes substituted phenolic disinfectants.[10,20-22]

For the Sterile Pack products, the entire double-bagged product is subjected to a sterilizing dose of gamma radiation, thus the contents inside the outer bag are sterile.[23] This allows the inner bag to be aseptically removed and brought into an environmentally-controlled area without introducing contaminants. A third sterile bag is included as a transport device. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent the presence of environmental contaminants and not pre-existing microorganisms in the medium that may have been introduced during manufacture. The **RODAC** plates have a marked grid to facilitate counting organisms. The Sterile Pack **Finger Dab** Isolator plates are triple-bagged and are intended for sampling gloved hands.

Formulae

Difco™ Sabouraud Dextrose Agar

Approximate Formula* Per Liter		
Peptic Digest of Animal Tissue	5.0	g
Pancreatic Digest of Casein	5.0	g
Dextrose	40.0	g
Agar	15.0	g

BBL™ Sabouraud Dextrose Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Peptic Digest of Animal Tissue	5.0	g
Dextrose	40.0	g
Agar	15.0	g

Difco™ Sabouraud Dextrose Broth

Approximate Formula* Per Liter		
Peptic Digest of Animal Tissue	5.0	g
Pancreatic Digest of Casein	5.0	g
Dextrose	20.0	g

Difco™ Fluid Sabouraud Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	5.0	g
Dextrose	20.0	g

Difco™ Sabouraud Maltose Agar

Approximate Formula* Per Liter		
Enzymatic Digest of Casein	10.0	g
Maltose	40.0	g
Agar	15.0	g

Difco™ Sabouraud Maltose Broth

Approximate Formula* Per Liter		
Peptic Digest of Casein	10.0	g
Maltose	40.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ Sabouraud Dextrose Agar – 65 g;
   BBL™ Sabouraud Dextrose Agar – 65 g;
   Difco™ Sabouraud Dextrose Broth – 30 g;
   Difco™ Fluid Sabouraud Medium – 30 g;
   Difco™ Sabouraud Maltose Agar – 65 g;
   Difco™ Sabouraud Maltose Broth – 50 g.
   Mix thoroughly.
2. Heat the agar media with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating which could cause a softer medium.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[6-8]

For cosmetic, food or environmental monitoring samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[9-12]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using Sabouraud Dextrose Agar and Sabouraud Dextrose Broth.[6-8]

For cosmetic, food or environmental monitoring samples, refer to appropriate standard references for details on test methods using Sabouraud Dextrose Agar or Sabouraud Dextrose Broth.[9-12]

For pharmaceutical samples, refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products and performing microbial enumeration tests and the isolation of *Candida albicans* using Sabouraud Dextrose Agar and Sabouraud Dextrose Broth.[1]

For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. Incubate the containers at 25-30°C with increased humidity. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Liquefy the medium in pour tubes by heating in boiling water. Cool to 45-50°C and pour into sterile Petri dishes. Allow to solidify for a minimum of 30 minutes.

Prepared tubed slants primarily are intended for use with pure cultures for maintenance or other purposes. With prepared plates and **Mycoflask™** bottles, streak the specimen as soon as possible after it is received in the laboratory, using a sterile inoculating loop to obtain isolated colonies. Consult appropriate references for information about the processing and inoculation of specimens.[6-8]

For the Sterile Pack media, sample selected surfaces by firmly pressing the agar medium against the test area. Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample. Do not move plate laterally as this spreads contaminants over the agar surface making resolution of colonies difficult. Slightly curved surfaces may be sampled with a rolling motion.

Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.

Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

Expected Results

After sufficient incubation, the containers should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Transfer of growth from slants to plated media may be required in order to obtain pure cultures of fungi.

Examine containers for fungal colonies exhibiting typical color and morphology.[24] Biochemical tests and serological procedures should be performed to confirm findings.

In the RODAC procedure, colonies are counted (fewer than 200 colonies for accurate counts) and expressed as either the number of colonies per RODAC plate or the number of colonies per cm.[5,10,11] Criteria for cleanliness of equipment and environment (surfaces) can be developed by using a database derived from repeated routine sampling of specific sites.[25]

Subculture colonies of interest so that positive identification can be made by means of biochemical testing and/or microscopic examination of organism smears.

Limitation of the Procedure

Some fungi may be inhibited by the acidic pH of the medium and by the antimicrobics in the selective media.[5-7]

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Availability

Difco™ Sabouraud Dextrose Agar

BAM CCAM CMPH2 COMPF EP JP MCM9 USP

Cat. No.	210940	Dehydrated – 100 g†
	210950	Dehydrated – 500 g†
	211661	Dehydrated – 2 kg†
	210930	Dehydrated – 10 kg†

BBL™ Sabouraud Dextrose Agar

BAM CCAM CMPH2 COMPF EP JP MCM9 USP

Cat. No.	211584	Dehydrated – 500 g†
	211585	Dehydrated – 5 lb (2.3 kg)†

United States and Canada

Cat. No.	221180	Prepared Plates (Deep Fill) – Pkg. of 20*†
	221278	Prepared Plates (Deep Fill) – Ctn. of 100*†
	221235	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	297739	Prepared Plates (150 x 15 mm-style), Deep Fill – Pkg. of 24*
	221012	Prepared Slants (A Tubes) – Pkg. of 10*
	221013	Prepared Slants (A Tubes) – Ctn. of 100*
	297072	Prepared Slants (C Tubes) – Pkg. of 10*
	297479	Prepared Slants (C Tubes) – Ctn. of 100*
	297812	Prepared Pour Tubes, 20 mL – Pkg. of 10*
	296182	Prepared Pour Tubes, 20 mL – Ctn. of 100*
	221136	**Mycoflask**™ Bottles – Pkg. of 10*
	221137	**Mycoflask**™ Bottles – Ctn. of 100*
	297720	Transgrow-style Bottles – Ctn. of 100*
	295699	Prepared Bottles, 1 oz. – Ctn. of 100*

Europe

Cat. No.	254039	Prepared Plates – Pkg. of 20*
	254083	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251180	Prepared Plates – Pkg. of 20*

BBL™ Sabouraud Dextrose Agar with Chloramphenicol

MCM9

Cat. No.	221851	Prepared Plates (Deep Fill) – Pkg. of 20*
	221825	Prepared Slants (C Tubes) – Ctn. of 100*
	221314	**Mycoflask**™ Bottles – Pkg. of 10*
	221315	**Mycoflask**™ Bottles – Ctn. of 100*
	299098	Prepared Bottles, 500 mL – Pkg. of 10

Japan

Cat. No.	251813	Prepared Plates (60 mm) – Ctn. of 240*
	251524	Prepared **RODAC**™ Plates – Pkg. of 30*

BBL™ Sabouraud Dextrose Agar with Chloramphenicol and Cycloheximide

Cat. No.	297649	Prepared Slants – Pkg. of 10*

BBL™ Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin

MCM9

Cat. No.	296359	Prepared Plates – Pkg. of 20*

BBL™ Sabouraud Dextrose Agar with Lecithin and Polysorbate 80

Cat. No.	221233	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	222244	Sterile Pack **RODAC**™ SL Plates – Pkg. of 10*
	215224	Sterile Pack **RODAC**™ SL Plates – Ctn. of 100*
	292653	Isolator Pack, **Finger Dab**™ Prepared Plates (100 × 15 mm-style) – Pkg. of 10*
	292654	Isolator Pack, **Finger Dab**™ Prepared Plates (150 × 15 mm-style) – Pkg. of 5*

Difco™ Sabouraud Dextrose Broth

BAM EP JP USP

Cat. No.	238220	Dehydrated – 100 g†
	238230	Dehydrated – 500 g†
	238210	Dehydrated – 2 kg†

BBL™ Sabouraud Dextrose Broth

BAM EP JP USP

Cat. No. 215193 Prepared Bottles, 100 mL – Pkg. of 10†

Difco™ Fluid Sabouraud Medium

Cat. No. 264210 Dehydrated – 500 g

Difco™ Sabouraud Maltose Agar

Cat. No. 211020 Dehydrated – 500 g

Difco™ Sabouraud Maltose Broth

Cat. No. 242910 Dehydrated – 500 g

** Store at 2-8°C.*
† QC testing performed according to USP/EP/JP performance specifications.

Sabouraud Agar, Modified • Sabouraud Dextrose Agar, Emmons • Sabouraud Dextrose Agar, Emmons, with Antimicrobics

Intended Use

Sabouraud Agar, Modified (Emmons) and Sabouraud Dextrose Agar, Emmons are used in qualitative procedures for cultivation of dermatophytes and other pathogenic and nonpathogenic fungi from clinical and nonclinical specimens.

Sabouraud Dextrose Agar, Emmons is rendered selective by the addition of antimicrobial agents.

Summary and Explanation

Sabouraud Dextrose Agar was devised by Sabouraud for the cultivation of dermatophytes.[1] The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes, and inhibitory to contaminating bacteria in clinical specimens.[2] The acidic pH, however, also may inhibit some fungal species.[2-4] Emmons modified the original formulation by adjusting the pH close to neutral to increase the recovery of fungi and by reducing the dextrose content from 40 to 20 g/L.[4] The two base formulations offered differ in peptone content and amount of agar. The addition of antimicrobics further increases the selectivity of the medium.[3,4]

User Quality Control

Identity Specifications

Difco™ Sabouraud Agar, Modified

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Sabouraud Agar, Modified

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours, or up to 7 days if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	Undiluted	Good
Candida albicans	10231	30-300	Good
Lactobacillus rhamnosus	9595	30-300	Good
Saccharomyces cerevisiae	9763	30-300	Good
Trichophyton mentagrophytes	9533	Undiluted	Good

Principles of the Procedure

Peptones are sources of nitrogenous growth factors. Dextrose provides an energy source for the growth of microorganisms. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria. Chloramphenicol is inhibitory to a wide range of gram-negative and gram-positive bacteria, and cycloheximide is an antifungal agent that is primarily active against saprophytic fungi and does not inhibit yeasts or dermatophytes.[5]

Formula

Difco™ Sabouraud Agar, Modified

Approximate Formula* Per Liter		
Enzymatic Digest of Casein	10.0	g
Dextrose	20.0	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 50 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for information about the processing and inoculation of specimens.[2,3]

Prepared tubed slants primarily are intended for use with pure cultures for maintenance or other purposes.

For isolating fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. Incubate the plates at 25-30°C in an inverted position (agar side up) with increased humidity. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C.

All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Expected Results

After sufficient incubation, the plates or tubes should show growth with or without isolated colonies. Transfer of growth from tubes to plated media may be required in order to obtain pure cultures of fungi.

Examine plates or tubes for fungal colonies exhibiting typical color and morphology.[6] Biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi.

References

1. Sabouraud. 1892. Ann. Dermatol. Syphil. 3:1061.
2. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.
3. LaRocco. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
5. Lorian (ed.). 1996. Antibiotics in laboratory medicine, 4th ed. Williams & Wilkins, Baltimore, Md.
6. Larone. 1995. Medically important fungi: a guide to identification, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Sabouraud Agar, Modified

SMWW

Cat. No.	274720	Dehydrated – 500 g
	274710	Dehydrated – 2 kg

BBL™ Sabouraud Dextrose Agar, Emmons

CMPH2 MCM9 SMWW

Cat. No.	221849	Prepared Plates (Deep Fill) – Pkg. of 20*
	221867	Prepared Plates (Deep Fill) – Ctn. of 100*
	221826	Prepared Slants (C Tubes) – Pkg. of 10
	221827	Prepared Slants (C Tubes) – Ctn. of 100
	296308	**Mycoflask™** Bottles – Pkg. of 10

BBL™ Sabouraud Dextrose Agar, Emmons with Chloramphenicol

MCM9

Cat. No.	297931	Prepared Plates (Deep Fill) – Pkg. of 10*
	297474	Prepared Plates (Deep Fill) – Ctn. of 100*

BBL™ Sabouraud Dextrose Agar, Emmons with Chloramphenicol and Cycloheximide

Cat. No.	297932	Prepared Plates (Deep Fill) – Pkg. of 10*

BBL™ Sabouraud Dextrose Agar, Emmons with Gentamicin

Cat. No.	296348	Prepared Plates (Deep Fill) – Pkg. of 20*

**Store at 2-8°C.*

Sabouraud Liquid Broth Modified

(See Antibiotic Assay Media)

Saline, 0.45% • Saline, Normal

Intended Use

Saline, 0.45% is used in procedures that require this saline diluent, such as with the VITEK™* identification and susceptibility testing system.

Saline, Normal (physiological) is used in procedures that require the use of an isotonic diluent.

**VITEK is a trademark of bioMérieux, Inc.*

Summary and Explanation

Saline, 0.45%, is used in procedures where this concentration of sodium chloride is suitable.[1]

An isotonic diluent may be used for dilution of bacterial cells to provide a concentration suitable for microscopic observation, determination of cell numbers, analysis for genetic or metabolic properties, washing cells preparatory to study, or preparation of standardized inocula.[2,3]

Principles of the Procedure

Saline is routinely used as a diluent to adjust the turbidity of bacterial cell suspensions to help maintain cell integrity and viability.[3,4]

Procedure

Using a sterile pipette, adjust the turbidity of a culture to be equivalent to a standard, such as a McFarland (barium sulfate) turbidity standard.[3,4]

The diluted culture should be used within the time limit stated in the appropriate method or procedure.

Expected Results

Cell integrity and viability is maintained within the parameters of the particular procedure being employed.

References

1. Aldridge, Jones, Gibson, Lanham, Meyer, Vannest and Charles. 1977. J. Clin. Microbiol. 6:406.
2. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.
3. Clinical and Laboratory Standards Institute. 2006. Approved standard M2-A9, Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.
4. Clinical and Laboratory Standards Institute. 2006. Approved standard M7-A7, Methods for dilution susceptibility tests for bacteria that grow aerobically, 7th ed. CLSI, Wayne, Pa.

Availability

BBL™ Saline, 0.45%

Cat. No.	299489	Prepared Tubes (K Size), 1 mL – Ctn. of 100

BBL™ Saline, Normal

Cat. No.	297815	Prepared Tubes (K Size), 1 mL – Ctn. of 100
	221818	Prepared Tubes (K Size), 5 mL – Pkg. of 10
	221819	Prepared Tubes (K size), 5 mL – Ctn. of 100
	295771	Prepared Tubes (C size), 5 mL – Ctn. of 100
	297753	Prepared Tubes (D size), 10 mL – Ctn. of 100

Europe

Cat. No.	257255	Prepared Tubes, 10 mL – Pkg. of 50

Salt Broth, Modified

Intended Use

Salt Broth, Modified is used to differentiate *Enterococcus* species from the *Streptococcus bovis* group of streptococci.

Summary and Explanation

Broth media containing 6.5% NaCl are used to differentiate enterococci by determining the salt tolerance of bile-esculin positive, catalase-negative gram-positive cocci.[1] Growth (turbidity) within 72 hours indicates that the strain is salt tolerant (i.e., positive), and it can be identified as an *Enterococcus* if it is also bile-esculin positive or if it is serologically group D.

Growth in Salt Broth, Modified will sometimes change the indicator color from purple to yellow within 72 hours.

Principles of the Procedure

Digests of animal tissue provide nitrogen and carbon compounds essential for bacterial growth. Dextrose is an energy source. Bromcresol purple is an indicator of acid production during bacterial metabolism. Sodium chloride serves as a selective and differential agent.

Enterococci are resistant to the high salt content, which in sensitive strains interferes with membrane permeability, and osmotic and electrokinetic equilibria.[2]

Procedure

Inoculate two or three streptococcal colonies into a tube of the medium. Incubate tubes with loosened caps for 72 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Examine tubes for growth (indicated by turbidity and sometimes change in the color of the indicator) at 24, 48 and 72 hours. Growth within 72 hours indicates that the strain is salt tolerant (i.e., positive). A change in color from purple to yellow also may occur.

References

1. Facklam, Sahm and Teixeira. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Salt Broth, Modified

Cat. No.	297189	Prepared Tubes (K size), 2 mL – Ctn. of 100
	221820	Prepared Tubes (K size), 5 mL – Pkg. of 10
	295792	Prepared Tubes (D size), 10 mL – Ctn. of 100

Schaedler Media
Schaedler Agar • Schaedler Agar with Vitamin K_1 and 5% Sheep Blood • Schaedler K-V Agar with 5% Sheep Blood • Schaedler Broth • Schaedler Broth with Vitamin K_1

Intended Use

Schaedler Agar is a base for several media formulations used for the recovery of anaerobic microorganisms.

Schaedler Agar with Vitamin K_1 and 5% Sheep Blood is used for the isolation and cultivation of fastidious aerobes and anaerobes from a variety of clinical and nonclinical specimens. It is especially useful for the recovery of the fastidious anaerobic bacteria such as *Bacteroides*, *Prevotella* and *Porphyromonas* species. Schaedler K-V Agar with 5% Sheep Blood, containing kanamycin and vancomycin, is especially useful in the selective isolation of *Bacteroides* and *Prevotella* species.

Schaedler Broth and Schaedler Broth with Vitamin K_1 are media used for the cultivation of fastidious aerobic and anaerobic microorganisms.

Summary and Explanation

In 1965, Schaedler, Dubos and Costello[1] reported on the bacterial flora of the gastrointestinal tract of mice. In these studies, several new media formulations were introduced. The majority of these contained inhibitors of specific bacterial species or groups since the authors indicated the need for selective media when processing specimens which contain large numbers of a heterogeneous bacterial population. The basal medium, without inhibitors, is the original version of the medium designated as Schaedler Agar. It was formulated to support the growth of fastidious anaerobic microorganisms such as lactobacilli, streptococci, clostridia and *Bacteroides*.

Mata and coworkers,[2] studying the fecal microflora in healthy persons in Central America, modified Schaedler Agar to produce a number of new formulations. The modifications in the basal medium of Schaedler included adjustments in the peptone content, since **Trypticase**™ Soy Broth was substituted for the **Trypticase** peptone component of the original formulation, and an increase in the sodium chloride content. Additionally, the dextrose concentration was reduced to avoid interference with hemolytic reactions and the yeast extract level lowered to avoid darkening of the medium.[3]

The inclusion of vitamin K_1, is an additional modification and was added since it is a growth requirement for some strains of *Prevotella melaninogenica* (*Bacteriodes melaninogenicus*)[4] and is reported to enhance the growth of some strains of *Bacteroides* and gram-positive nonsporeformers.[5]

The combination of kanamycin and vancomycin in Schaedler K-V Agar with 5% Sheep Blood is used for the selective isolation of gram-negative anaerobes.[6]

Schaedler Broth has the same formula as Schaedler Agar except that the agar is omitted. Stalons et al.[7] found Schaedler Broth to be the most effective medium of nine broth media tested for the growth of obligately anaerobic bacteria when incubated in an anaerobic atmosphere. The incorporation of vitamin K_1 broadens the spectrum of organisms that can be cultivated in Schaedler Broth.

Principles of the Procedure

The combination of three peptones derived from both animal and vegetable sources, dextrose and yeast extract render the basic formulation highly nutritious by providing nitrogenous growth factors, carbohydrates as energy sources and vitamins. The sheep blood and hemin also are important in stimulating the growth of fastidious microorganisms. As discussed above, the vitamin K_1 additive is crucial for the recovery of certain anaerobes.

The addition of the antimicrobial agents kanamycin and vancomycin in the agar medium renders the medium selective for gram-negative microorganisms. The kanamycin inhibits protein synthesis in susceptible organisms, whereas the vancomycin inhibits gram-positive bacteria by interfering with cell wall synthesis.[8]

Using Schaedler media, fastidious aerobes and anaerobes grow well; however, the type of organisms recovered is dependent on the environment utilized in the incubation process (aerobic, aerobic supplemented with carbon dioxide or anaerobic conditions).

Formulae

BBL™ Schaedler Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	8.2	g
Peptic Digest of Animal Tissue	2.5	g
Papaic Digest of Soybean Meal	1.0	g
Dextrose	5.8	g
Yeast Extract	5.0	g
Sodium Chloride	1.7	g
Dipotassium Phosphate	0.8	g
L-Cystine	0.4	g
Hemin	0.01	g
Tris (hydroxymethyl) aminomethane	3.0	g
Agar	13.5	g

BBL™ Schaedler Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   BBL™ Schaedler Agar – 41.9 g;
   BBL™ Schaedler Broth – 28.4 g.
   Mix thoroughly.
2. If desired, add 1 mL of a 1% vitamin K_1 solution in absolute ethanol.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Autoclave at 121°C for 15 minutes.
5. For the agar medium, cool to approximately 45°C and add 5% sterile defibrinated sheep blood when required.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Agars

These media should be reduced immediately prior to inoculation by placing them under anaerobic conditions for 18-24 hours.[9]

Use standard procedures to obtain isolated colonies from specimens. Inoculate an enrichment broth, such as Enriched Thioglycollate Medium, at the same time as the primary plates to detect small numbers of anaerobes.

Incubate plates and tubes immediately after inoculation, with plates in an inverted position (agar side up) under anaerobic conditions at 35°C, or place the media in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates and tubes is accumulated (no longer than 3 hours). Incubate for at least 48 hours and, if no growth occurs, continue incubation for up to 7 days. It is recommended that an indicator of anaerobiosis be used.

User Quality Control

Identity Specifications

BBL™ Schaedler Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.19% solution, soluble in purified water upon boiling. Solution is medium, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Medium, tan to yellow, clear to slightly hazy.
Reaction of 4.19% Solution at 25°C:	pH 7.6 ± 0.2

BBL™ Schaedler Broth

Dehydrated Appearance:	Fine, homogeneous, may contain tan specks.
Solution:	2.84% solution, soluble in purified water upon boiling. Solution is light to medium, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Light to medium, tan to yellow, clear to slightly hazy.
Reaction of 2.84% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

BBL™ Schaedler Agar

Prepare the medium per label directions without (plain) and with added vitamin K_1 and 5% sheep blood (SB). Inoculate and incubate anaerobically at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH VIT. K_1 AND SB
Bacteroides fragilis	25285	$\leq 10^6$	N/A	Good
Clostridium perfringens	13124	$\leq 10^6$	N/A	Good, beta hemolysis
Streptococcus pyogenes	19615	10^3-10^4	Good	N/A

BBL™ Schaedler Broth

Prepare the medium per label directions. Inoculate and incubate anaerobically at 35 ± 2°C for up to 7 days (incubate *S. pyogenes* aerobically).

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Bacteroides vulgatus	8482	$\leq 10^3$	Growth
Clostridium novyi A	7659	$\leq 10^3$	Growth
Streptococcus pyogenes	19615	$\leq 10^3$	Growth

Examine the selective medium for growth after 48 hours of incubation. Cultures should not be regarded as negative until after 7 days incubation. Since some anaerobes may be inhibited due to the selective nature of the medium, it is advisable to include a nonselective medium such as Schaedler Agar with Vitamin K_1 and 5% Sheep Blood.

Broths

Inoculate the specimen directly into the broth medium. Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours prior to use. Alternatively, liquid media may be reduced immediately prior to use by boiling with caps loosened and cooling with tightened caps to room temperature before inoculation.

Incubate tubes at 35 ± 2°C in the appropriate atmosphere (aerobic, anaerobic, or supplemented with carbon dioxide) for up to 7 days.

Expected Results

Agars

In order to determine the relationship to oxygen of each colony type present on the medium, follow established procedures.[10] The colony types that prove to contain obligate anaerobes can be further studied.[11]

Broths

Growth in tubes is indicated by the presence of turbidity compared to an uninoculated control. If growth appears, cultures should be examined by Gram stain and subcultured onto appropriate media (e.g., a TSA II and/or Chocolate II Agar plate, etc.). If obligate anaerobes are suspected, subcultures should be incubated anaerobically.

References

1. Schaedler, Dubos and Costello. 1965. J. Exp. Med. *122*:59.
2. Mata, Carrillo and Villatoro. 1969. Appl. Microbiol. *17*:596.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Gibbons and MacDonald. 1960. J. Bacteriol. *80*:164.
5. Finegold, Sutter, Attebery and Rosenblatt. 1974. *In* Lennette, Spaulding and Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
6. Finegold, Miller and Posnick. 1965. Ernahrungsforschung *10*:517.
7. Stalons, Thornsberry and Dowell. 1974. Appl. Microbiol. *27*:1098.
8. Estevez. 1984. Lab. Med. *15*:258.
9. Dowell. 1975. *In* Balows (ed.). Clinical microbiology. How to start and when to stop. Charles C. Thomas, Springfield, Ill.
10. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
11. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Schaedler Agar

Cat. No. 212189 Dehydrated – 500 g

BBL™ Schaedler Agar with Vitamin K_1 and 5% Sheep Blood

United States and Canada

Cat. No. 221539 Prepared Plates – Pkg. of 20*
221540 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254042 Prepared Plates – Pkg. of 20*
254084 Prepared Plates – Ctn. of 120*

BBL™ Schaedler K-V Agar with 5% Sheep Blood

United States and Canada

Cat. No. 221555 Prepared Plates – Pkg. of 20*
221556 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254023 Prepared Plates – Pkg. of 20*
254077 Prepared Plates – Ctn. of 120*

BBL™ Schaedler Broth

Cat. No. 212191 Dehydrated – 100 g

BBL™ Schaedler Broth with Vitamin K_1

Cat. No. 221541 Prepared Tubes – Pkg. of 10*
221542 Prepared Tubes – Ctn. of 100*

**Store at 2-8°C.*

Selective Seven H11 Agar

(See Seven H11 Agars)

Selective Streptococcus Agar

Intended Use

Selective Streptococcus Agar is designed for the isolation of group A streptococci from respiratory sources.

Summary and Explanation

Roantree et al.[1] introduced a medium for isolation of group A beta-hemolytic streptococci. The medium enriched with yeast nucleic acid and maltose promoted increased colony size and enhanced clarity and sharpness of hemolytic zones produced by these organisms.[2,3]

Principles of the Procedure

Selective Streptococcus Agar is prepared from beef extract and casein peptone, which are relatively free of dextrose, permitting the addition of animal blood to detect hemolytic activity. The incorporation of the antimicrobial agents, neomycin and polymyxin B, provides suppression of normal throat flora for improved recovery of *Streptococcus pyogenes*.

Procedure

To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface, and streak the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs may be streaked onto the medium with a sterilized inoculating loop. Without resterilizing the loop, stab the agar two or three times in the area of heaviest inoculation. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Incubate the plates in an inverted position (agar side up) at 35-37°C for 18-48 hours in a CO_2-enriched atmosphere.

Expected Results

After 18-48 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Group A streptococci (*S. pyogenes*) will appear as translucent or opaque, white to gray, small colonies surrounded by a zone of beta hemolysis. Most *Neisseria* species and gram-negative rods are inhibited.

References

1. Roantree, Rantz and Haines. 1958. J. Lab. Clin. Med. *52*:496.
2. Bernheimer and Rodbart. 1948. J. Exp. Med. *88*:149.
3. Watner, Bestakova and Motilkova. 1955. Schweiz Ztschr. Allg. Path. *18*:278.

Availability

BBL™ Selective Streptococcus Agar

United States and Canada

Cat. No.	221934	Prepared Plates – Pkg. of 20*
	221935	Prepared Plates – Ctn. of 100*

Japan

Cat. No.	212498	Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Selenite Broth • Selenite-F Broth

Intended Use

Selenite Broth (Selenite-F Broth) is used as an enrichment medium for the isolation of *Salmonella* from feces, urine, water, foods and other materials of sanitary importance.

Summary and Explanation

Selenite Broth was devised by Leifson,[1] who demonstrated that selenite was inhibitory for coliforms and certain other microbial species, such as fecal streptococci, present in fecal specimens and, thus, was beneficial in the recovery of *Salmonella* species. He found that the inhibited strains would eventually break through, but if subcultures were made from the enrichment broth after 8-12 hours of incubation, the isolation of *Salmonella* was possible without overwhelming growth of many members of the intestinal flora.

Enrichment media are routinely employed for detection of pathogens in fecal specimens since the pathogens usually represent only a small percentage of the intestinal flora. Selenite Broth and the related medium, Selenite Cystine Broth, are recommended for use in the recovery of *Salmonella* with subcultures being made after 12-18 hours of incubation. For detection of *Shigella*, GN Broth is a satisfactory enrichment medium.[2] Campylobacter Thioglycollate Medium with 5 Antimicrobics is recommended for specimens suspected to contain *Campylobacter jejuni*.[3]

Principles of the Procedure

The peptone provides essential nitrogenous and carbon compounds. The lactose in the medium serves to maintain a uniform pH. When selenite is reduced by the growth of bacteria, alkali is produced, and such increase in pH would lessen the toxicity of the selenite and result in overgrowth of extraneous bacteria. The acid produced by lactose fermentation serves to maintain a neutral or slightly decreased pH. The function of the phosphate is two-fold; it serves to maintain a stable pH and

User Quality Control

Identity Specifications

Difco™ Selenite Broth

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is very light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Selenite Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, subculture onto MacConkey Agar plates and incubate plated media at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONIES ON MACCONKEY AGAR
Escherichia coli	25922	10^2-10^3	Partial inhibition	Pink with bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Colorless

lessens the toxicity of the selenite, thus increasing the capacity of the medium. Sodium selenite inhibits many species of gram-positive and gram-negative bacteria including enterococci and coliforms.

Formula

Difco™ Selenite Broth

Approximate Formula* Per Liter

Pancreatic Digest of Casein	5.0	g
Lactose	4.0	g
Sodium Selenite	4.0	g
Sodium Phosphate	10.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water.
2. Heat to boiling. Avoid overheating. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For feces and other solid materials, suspend 1-2 g of the specimen in the broth (approximately 10-15% by volume) and emulsify with an inoculating needle, if necessary.

Incubate tubes with loosened caps at 35 ± 2°C for up to 24 hours. Subcultures should be made after 12-18 hours of incubation, if possible. Coliforms will tend to overgrow the pathogens if incubated longer than 24 hours.

Expected Results

After incubation, there should be an increase in the number of pathogens that the medium is designed to select for and enrich. Subculture onto appropriate selective and differential media (e.g., MacConkey Agar, Hektoen Enteric Agar, XLD Agar, XLT4 Agar, **CHROMagar**™ Salmonella) to isolate pathogens for identification.

Limitation of the Procedure

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult references for detailed information and recommended procedures.[3]

References

1. Leifson. 1936. Am. J. Hyg. *24*:423.
2. Taylor and Harris. 1965. Am. J. Clin. Pathol. *44*:476.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Selenite Broth

BS12 MCM9 SMWW

Cat. No. 227540 Dehydrated – 500 g

BBL™ Selenite-F Broth

BS12 MCM9 SMWW

Cat. No. 221020 Prepared Tubes (K Tubes), 8 mL – Pkg. of 10*
221021 Prepared Tubes (K Tubes), 8 mL – Ctn. of 100*

**Store at 2-8°C.*

Selenite Cystine Broth

Intended Use

Selenite Cystine Broth is used as a selective enrichment medium for the isolation of *Salmonella* from feces, foods, water and other materials of sanitary importance.

Summary and Explanation

Leifson found that selenite inhibited fecal streptococci and coliforms during the first 8-12 hours of incubation, thereby permitting salmonellae to replicate without overwhelming interference from other members of the intestinal flora.[1] North and Bartram modified Leifson's Selenite-F Enrichment broth by adding cystine, which stimulated growth of *Salmonella*.[2]

User Quality Control

Identity Specifications

Difco™ Selenite Cystine Broth

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is very light amber, may have a trace tint of pink orange, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Selenite Cystine Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 ± 2 hours. After incubation, subculture onto MacConkey Agar plates and incubate plated media at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONIES ON MACCONKEY AGAR
Escherichia coli	25922	10^2-10^3	Partial to complete inhibition	Pink with bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Colorless
Shigella sonnei	9290	10^2-10^3	Fair to Good	Colorless

The cystine-containing formulation is recommended by the Food and Drug Administration, AOAC International and American Public Health Association for detecting *Salmonella* in foods and waters.[3-6]

Selenite Cystine Broth and similar enrichment media are useful for detecting *Salmonella* in the nonacute stages of illness when the organisms occur in the feces in low numbers and for epidemiological studies to enhance the detection of low numbers of organisms from asymptomatic or convalescent patients.[7]

Principles of the Procedure

Peptone provides amino acids and other nitrogenous substances. Lactose provides a source of energy, and sodium phosphate buffers the medium to maintain the pH. Sodium selenite inhibits gram-positive bacteria and suppresses the growth of most gram-negative enterics other than *Salmonella*. L-cystine is incorporated to improve the recovery of *Salmonella*.[8]

Formula

Difco™ Selenite Cystine Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Lactose	4.0	g
Sodium Phosphate	10.0	g
Sodium Selenite	4.0	g
L-Cystine	0.01	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water.
2. Heat to boiling. Avoid overheating. DO NOT AUTOCLAVE.
3. Use immediately.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For feces, food samples or other solid materials, suspend 1-2 g of the specimen in the broth (approximately 10-15% by volume) and emulsify with an inoculating needle, if necessary. Consult references for information about the processing and inoculation of other samples or specimens.[3-6]

Incubate the tubes at 35°C and subculture onto selective and differential media (e.g., MacConkey Agar, XLD Agar, XLT4 Agar, **CHROMagar**™ Salmonella) after 6-8 hours of incubation and again after 12-24 hours of incubation.

Expected Results

After incubation, the number of colonies of pathogens the medium is designed to select should increase. Subculture onto appropriate selective and differential media to isolate pathogens for identification.

Limitations of the Procedure

1. Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult references for detailed information and recommended procedures.[3,5]
2. A brick red precipitate may appear if the medium is overheated during preparation or exposed to excessive moisture during storage.

References

1. Leifson. 1936. Am. J. Hyg. *24*:423.
2. North and Bartram. 1953. Appl. Microbiol. *1*:130.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
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8. Chapin and Murray. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Selenite Cystine Broth

AOAC BAM CCAM COMPF ISO SMD SMWW

Cat. No.	268740	Dehydrated – 500 g
	268710	Dehydrated – 2 kg

BBL™ Selenite Cystine Broth

AOAC BAM CCAM COMPF ISO SMD SMWW

Cat. No. 297711 Prepared Tubes (A Tubes), 20 mL – Ctn. of 100*

**Store at 2-8°C.*

Serum Tellurite Agar

Intended Use

Serum Tellurite Agar is a selective and differential medium used for isolation of members of the genus *Corynebacterium*, particularly in the laboratory diagnosis of diphtheria.

Summary and Explanation

Serum Tellurite Agar, which contains lamb serum and potassium tellurite, was developed for use in the examination of nose, throat and vaginal cultures for isolation of *Corynebacterium* species.[1]

Principles of the Procedure

The nutrients in Serum Tellurite Agar are supplied by casein and meat peptones, which provide growth factors such as nitrogen, carbon, sulfur, and trace ingredients. Dextrose is an energy source. Sodium chloride maintains osmotic equilibrium.

Corynebacterium diphtheriae ATCC™ 9675

Potassium tellurite is inhibitory for a variety of microorganisms; however, corynebacteria are resistant to tellurite and produce characteristic gray to black colonies on media containing tellurite.[2] Lamb serum contains growth factors required by *Corynebacterium* species.

Procedure

Inoculate plates and/or slants with specimen material. Incubate tubes with loosened caps for 24-72 hours at 35 ± 2°C in an aerobic atmosphere. The slanted medium is also convenient for use in cultivation of pure cultures for use in laboratory studies. Incubate plates for 24-48 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Colonies which are gray to black in color are presumptively identified as *Corynebacterium* species. These colonies should be picked and used to obtain the organism in pure culture prior to organism speciation.

Typical colonial morphology on Serum Tellurite Agar is as follows:

Corynebacteria	Gray, dark centers, smooth to rough, flat
Streptococci	Small, white to gray
Staphylococci	Large, white to gray
Micrococci	Large, white to gray, rough
Candida	Small, white to gray
Listeria monocytogenes	Gray
Gram-negative bacteria	No growth to trace growth

References

1. Albers. 1947. U.S. Naval Med. Bull. 47:33.
2. Funke and Bernard. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.). Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Serum Tellurite Agar

BS12 CMPH2 MCM9

Cat. No.	221183	Prepared Plates – Pkg. of 20*
	221024	Tubed Slants – Pkg. of 10*

**Store at 2-8°C.*

Seven H11 Agars
Mycobacteria 7H11 Agar • Seven H11 Agar Base
Seven H11 Agar • Selective Seven H11 Agar
Seven H11 Agar with Aspartic Acid and Pyruvate

Intended Use

These media are used in qualitative procedures for isolation and cultivation of mycobacteria, especially *Mycobacterium tuberculosis*, from clinical and nonclinical specimens.

Summary and Explanation

Seven H11 Agar (also referred to as Middlebrook 7H11 Agar) was developed by Cohn et al. by the addition of casein hydrolysate to 7H10 Agar.[1] Seven H11 Agar provides enhanced

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Mycobacteria 7H11 Agar

Dehydrated Appearance:	Light beige to light beige with green tint, free-flowing, homogeneous.
Solution:	2.1 g/90 mL solution, soluble in purified water with 0.5% glycerol upon boiling. Solution is light yellowish green, very slightly to slightly opalescent.
Prepared Appearance:	Light yellowish green, very slightly to slightly opalescent.
Reaction of 2.1 g/90 mL with 0.5% Glycerol Solution at 25°C:	pH 6.6 ± 0.2

Identity Specifications

BBL™ Seven H11 Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	19 g/900 mL solution, soluble in purified water with 0.5% glycerol. Solution is colorless to pale tan to light tan-green, slightly hazy to hazy.
Prepared Appearance:	Colorless to pale tan to light tan-green, slightly hazy to hazy.
Reaction of 19 g/900 mL with 0.5% Glycerol Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Difco™ Mycobacteria 7H11 Agar

Prepare the medium per label directions with added Middlebrook OADC enrichment. Inoculate and incubate at 35 ± 2°C with 3-5% and up to 10% CO_2 for up to 21 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-2×10^3	Partial inhibition
Mycobacterium tuberculosis H37Ra	25177	3×10^2-10^3	Good
Mycobacterium kansasii Group I	12478	3×10^2-10^3	Good
Mycobacterium scrofulaceum Group II	19981	3×10^2-10^3	Good
Mycobacterium intracellulare Group III	13950	3×10^2-10^3	Good
Mycobacterium fortuitum Group IV	6841	3×10^2-10^3	Good

Cultural Response

BBL™ Seven H11 Agar Base

Prepare the medium per label directions with added Middlebrook OADC enrichment. Inoculate and incubate at 35 ± 2°C with 3-5% and up to 10% CO_2 for up to 21 days.

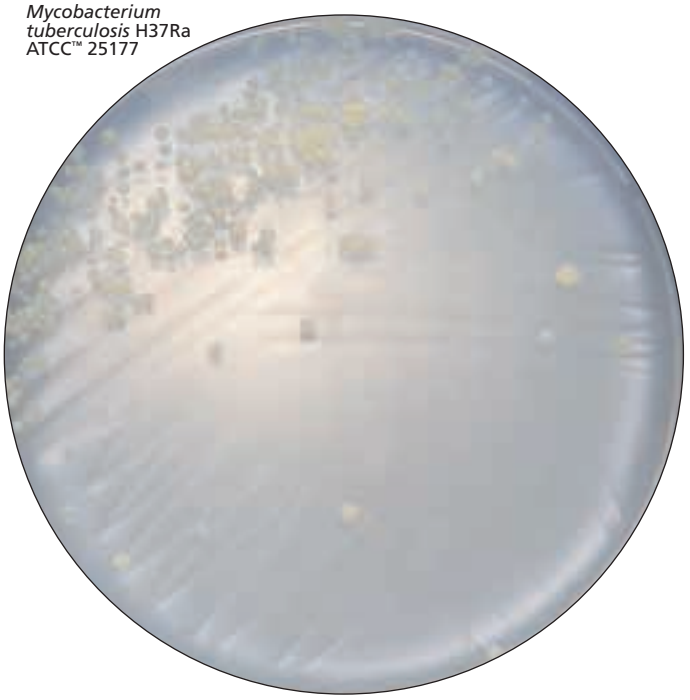
ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Mycobacterium tuberculosis H37Ra	25177	10^3	Good
Mycobacterium kansasii Group I	12478	10^2	Good
Mycobacterium scrofulaceum Group II	19981	10^2	Good
Mycobacterium intracellulare Group III	13950	10^2	Good
Mycobacterium fortuitum Group IV	6841	10^2	Good

growth of fastidious, drug-resistant strains of *M. tuberculosis* that grow poorly (or not at all) on 7H10 Agar or other widely-used media.[1,2]

The Selective Seven H11 Agar is 7H11 Agar modified by the addition of four antimicrobial agents: polymyxin B, carbenicillin, amphotericin B and trimethoprim lactate. Mitchison et al. initially developed the medium to reduce the need for decontamination procedures.[3] They found that the alkaline agents used to reduce the growth of contaminating organisms inhibited some species of mycobacteria. McClatchy recommended reducing the concentration of carbenicillin used by Mitchison et al. to make the medium less inhibitory to mycobacteria.[4]

The addition of pyruvate to Seven H11 Agar has been recommended for specimens suspected of containing *Mycobacterium bovis*. The addition of aspartic acid has been recommended to enhance the production of niacin.[5]

Deep-filled plates are available to reduce the effects of drying during prolonged incubation.



Mycobacterium tuberculosis H37Ra
ATCC™ 25177

Principles of the Procedure

Middlebrook 7H10 Agar is a defined medium consisting of oleic acid-albumin enrichment, glycerol, dextrose and inorganic

compounds to supply the nutrients necessary to support the growth of mycobacterial species. Catalase destroys toxic peroxides that may be present in the medium. Malachite green acts as an inhibitory agent to provide partial inhibition of contaminating bacteria.

Seven H11 Agar consists of 7H10 Agar supplemented with pancreatic digest of casein to enhance the growth of fastidious strains of *M. tuberculosis*.

The addition of antimicrobial agents to Seven H11 Agar improves the recovery of mycobacteria from specimens containing mixed flora.[2] Polymyxin B is a polypeptide antibiotic that selectively inhibits most species of gram-negative bacilli, including *Pseudomonas*, but not *Proteus* species.[6] Carbenicillin is a semi-synthetic penicillin effective against gram-positive and gram-negative bacteria, including strains of *Escherichia coli* resistant to other antimicrobial agents.[6] Amphotericin B is an antifungal antibiotic, and trimethoprim lactate is a synthetic antimicrobial agent that inhibits both gram-positive and gram-negative bacteria, including *Proteus* species.

With Seven H11 Agar containing aspartic acid and pyruvate, aspartic acid serves as a precursor for niacin synthesis by *M. tuberculosis* and *M. bovis*. All mycobacteria produce nicotinic acid (niacin). Because of a blocked metabolic pathway for the conversion of free niacin to nicotinic mononucleotide, *M. tuberculosis* accumulates niacin and excretes it into the culture medium, a function that differentiates it from most other mycobacterial species. Pyruvate enhances the recovery of *M. bovis*.

Formulae

Difco™ Mycobacteria 7H11 Agar

Approximate Formula* Per 900 mL		
Pancreatic Digest of Casein	1.0	g
L-Glutamic Acid	0.5	g
Sodium Citrate	0.4	g
Pyridoxine	1.0	mg
Biotin	0.5	mg
Ferric Ammonium Citrate	0.04	g
Ammonium Sulfate	0.5	g
Disodium Phosphate	1.5	g
Monopotassium Phosphate	1.5	g
Magnesium Sulfate	0.05	g
Agar	15.0	g
Malachite Green	1.0	mg

BBL™ Seven H11 Agar Base

Approximate Formula* Per 900 mL		
Pancreatic Digest of Casein	1.0	g
Monosodium Glutamate	0.5	g
Sodium Citrate	0.4	g
Pyridoxine	1.0	mg
Biotin	0.5	mg
Ferric Ammonium Citrate	0.04	g
Ammonium Sulfate	0.5	g
Disodium Phosphate	1.5	g
Monopotassium Phosphate	1.5	g
Magnesium Sulfate	0.05	g
Agar	13.5	g
Malachite Green	0.25	mg
Zinc Sulfate	1.0	mg
Copper Sulfate	1.0	mg
Calcium Chloride	0.5	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precaution[7]

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 900 mL of purified water containing 5 mL of glycerol:
   Difco™ Mycobacteria 7H11 Agar – 21 g;
   BBL™ Seven H11 Agar Base – 19 g.
2. Swirl to obtain a smooth suspension. For the **Difco** base, boil if necessary to completely dissolve the powder. For the **BBL** base do not boil.
3. Autoclave at 121°C for 15 minutes for the **Difco** base and 10 minutes for the **BBL** base.
4. Aseptically add 100 mL of Middlebrook OADC Enrichment to the medium when cooled to 50-55°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria. N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. These reagents are provided in the **BBL™ MycoPrep™** Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[8-11]

Following inoculation keep tubes shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C.

Slanted media should be incubated in a horizontal plane until the inoculum is absorbed. Tubes should have screw caps loose for the first 3 weeks to permit circulation of carbon dioxide for the initiation of growth. Thereafter, to prevent dehydration, tighten caps; loosen briefly once a week. Stand tubes upright if space is a problem.

NOTE: Cultures from skin lesions suspected to be *M. marinum* or *M. ulcerans* should be incubated at 25-33°C for primary isolation; cultures suspected to contain *M. avium* or *M. xenopi* exhibit optimum growth at 40-42°C.[4] Incubate a duplicate culture at 35-37°C.

For information on the niacin test, consult the *BBL™ Quality Control and Product Information Manual for Plated and Tubed Media* and other appropriate references.[2,8-11] **BBL™ Taxo™** TB Niacin Test Reagents (strips and control) may be used instead of the test reagents.

Expected Results

Cultures on Seven H11 Agar should be read within 5-7 days after incubation and once a week thereafter for up to 8 weeks.

Record Observations:[8]

1. Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7 days. Slow growers require more than 7 days for mature colony forms.
2. Pigment production
   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.

Test all nonchromogenic mycobacteria on Seven H11 Agar with Aspartic Acid and Sodium Pyruvate for niacin production; only the rough nonchromogenic strains need to be tested for niacin. A culture must have at least 50-100 colonies with growth 3-4 weeks old. *M. tuberculosis* and the more rare *M. simiae* are usually niacin positive. Most other mycobacteria are niacin negative.

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

1. Cohn, Waggoner and McClatchy. 1968. Am. Rev. Respir. Dis. *98*:295.
2. Murray, Baron, Jogensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Mitchison, Allen, Carrol, Dickinson and Aber. 1972. J. Med. Mycol. *5*:165.
4. McClatchy, Waggoner, Kanes, Cernich and Bolton. 1976. Am. J. Clin. Pathol. *65*:412.
5. Kilburn, Stottmeier and Kubica. 1968. Am. J. Clin. Pathol. *50*:582.
6. Garrod and O'Grady. 1971. Antibiotics and chemotherapy, 3rd ed. Williams & Wilkins, Baltimore, Md.
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8. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS, Centers for Disease Control, Atlanta, Ga.
9. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
10. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.
11. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.

Availability

Difco™ Mycobacteria 7H11 Agar

Cat. No. 283810 Dehydrated – 500 g

BBL™ Seven H11 Agar Base

Cat. No. 212203 Dehydrated – 500 g

BBL™ Seven H11 Agar

BS12 CMPH2 MCM9

Cat. No. 221870 Prepared Plates (Deep Fill) – Pkg. of 10*
221391 Prepared Slants (A Tubes) – Pkg. of 10*
221392 Prepared Slants (A Tubes) – Ctn. of 100*
296105 Prepared Slants (C Tubes) – Pkg. of 10*
297704 Prepared Slants (C Tubes) – Ctn. of 100*

Japan
Cat. No. 252119 Prepared Plates (Deep Fill) – Pkg. of 20*

BBL™ Selective Seven H11 Agar

BS12 CMPH2 MCM9

Cat. No. 221868 Prepared Plates (Deep-fill) – Pkg. of 10*
297315 Prepared Slants (A Tubes) – Pkg. of 10*
297639 Prepared Slants (A Tubes) – Ctn. of 100*
297184 Prepared Slants (C Tubes) – Pkg. of 10*
297654 Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Seven H11 Agar with Aspartic Acid and Sodium Pyruvate

Cat. No. 221958 Prepared Slants (A Tubes) – Pkg. of 10*

BBL™ Middlebrook 7H11 Agar//Selective 7H11 Agar

BS12 CMPH2 MCM9

Cat. No. 297250 Prepared Bi-Plate Dishes – Pkg. of 20*

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

BBL™ Taxo™ TB Niacin Test Strips and Control

Cat. No. 231741 Vial – 25 strips*
231735 Cartridge, Control – 50 discs*

**Store at 2-8°C.*

Shigella Broth

Intended Use

Shigella Broth is a selective enrichment broth for the isolation of *Shigella* species from food.

Summary and Explanation

Shigella was first recognized as the etiologic agent of bacillary dysentery or shigellosis in the 1890s.[1] Humans are the only natural reservoir. No natural food products harbor endogenous *Shigella* species, but a wide variety of foods may be contaminated.[1]

User Quality Control

Identity Specifications

Difco™ Shigella Broth

Dehydrated Appearance:	Off-white to light tan, free-flowing, may appear moist, free of extraneous material.
Solution:	3.1% solution, soluble in purified water. Solution is pale to light amber, clear to slightly opalescent.
Prepared Appearance:	Pale to light amber, clear to slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Shigella Broth

Prepare the medium per label directions. Inoculate and incubate under anaerobic conditions at 40-44°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	100	Good
Shigella flexneri	12022	100	Good
Shigella sonnei	25931	100	Good

Shigellosis can manifest itself as a waterborne or a foodborne disease. It is usually spread among people by food handlers with poor personal hygiene. Foods most often incriminated in the transmission of the disease have been potato salad, shellfish, raw vegetables and Mexican food.[2]

The infectivity dose is extremely low. As few as ten *S. dysenteriae* bacilli can cause clinical disease, whereas 100-200 bacilli are needed for *S. sonnei* or *S. flexneri* infection.[1] One possible reason for this low-dose response may be that virulent *Shigellae* can withstand the low pH of gastric juice.[1]

Shigella species are gram-negative, nonmotile, facultatively anaerobic, non-sporeforming rods. They utilize glucose and other carbohydrates, producing acid but not gas. They do not decarboxylate lysine or ferment lactose. *Shigella* organisms may be difficult to distinguish biochemically from *E. coli*. The genus *Shigella* consists of four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*.

Common contaminating bacteria found in food sources could mask the presence of any *Shigella* that could be present in the sample. Identification of *Shigella* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Shigella Broth is based on the formula developed by Mehlman, Romero and Wentz.[2] Selectivity of the medium is achieved by the addition of novobiocin to the completed medium. Shigella Broth is recommended in standard test methods for use as a selective enrichment when isolating *Shigella* sp. from food samples.[3-6]

Principles of the Procedure

Tryptone is a source of carbon, nitrogen, vitamins and minerals. Phosphates are the buffering agents in the solution. Sodium chloride maintains the osmotic balance. Glucose is the carbohydrate source. Polysorbate 80 neutralizes preservatives in food products, allowing bacteria to grow. Novobiocin suppresses the growth of nuisance organisms commonly found in foods.

Formula

Difco™ Shigella Broth

Approximate Formula* Per Liter		
Tryptone	20.0	g
Dipotassium Phosphate	2.0	g
Monopotassium Phosphate	2.0	g
Sodium Chloride	5.0	g
Glucose	1.0	g
Polysorbate 80	1.5	g

**Adjusted and/or supplemented as required to meet performance criteria*

Directions for Preparation from Dehydrated Product

1. Dissolve 31.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
3. Prepare novobiocin solution by weighing 50 mg of novobiocin into 1 L of purified water. Sterilize by filtration using a 0.45µ filter.
4. Add 2.5 mL of sterile novobiocin solution from Step 3 to 225 mL of Shigella Broth.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[3-6]

Consult appropriate standard references for details on test methods using Shigella Broth.[3-6]

Expected Results

Growth is evident by the appearance of turbidity.

References

1. Sureshbabu, Poothirikovil, Abuhammour, and Burny. 2008. Shigella infection. <http://emedicine.medscape.com/article/968773>.
2. Mehlman, Romero and Wentz. 1985. J. Assoc. Off. Anal. Chem. 68:552.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Health Canada. The compendium of analytical methods, online. Food Directorate, Health Products and food Branch, Health Canada, Ottawa, Ontario Canada.
5. International Organization for Standardization. 2004 Microbiology of food and animal feeding stuffs – horizontal method for the detection of Shigella spp. ISO 21567, 2004-11-01. International Organization for Standardization, Geneva, Switzerland.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington. D.C.

Availability

Difco™ Shigella Broth

BAM CCAM COMPF ISO

Cat. No. 214915 Dehydrated – 500 g

Simmons Citrate Agar

Intended Use
Simmons Citrate Agar is used for the differentiation of gram-negative bacteria on the basis of citrate utilization.

Summary and Explanation
Koser,[1] in 1923, developed a liquid medium consisting of inorganic salts in which an ammonium salt was the only source of nitrogen and citrate was the sole carbon source in order to differentiate between what are now known as *Escherichia coli* and *Enterobacter aerogenes* as part of the IMViC (Indole-Methyl Red-Voges Proskauer-Citrate) reactions. Simmons,[2] in 1926, modified Koser's formulation with the addition of 1.5% agar and bromthymol blue.[3] Organisms capable of metabolizing citrate grow well on this medium.

Principles of the Procedure
Organisms able to utilize ammonium dihydrogen phosphate and sodium citrate as the sole sources of nitrogen and carbon, respectively, will grow on this medium and produce an alkaline reaction as evidenced by a change in the color of the bromthymol blue indicator from green (neutral) to blue (alkaline).

Formula
BBL™ Simmons Citrate Agar

Approximate Formula* Per Liter		
Ammonium Dihydrogen Phosphate	1.0	g
Dipotassium Phosphate	1.0	g
Sodium Chloride	5.0	g
Sodium Citrate	2.0	g
Magnesium Sulfate	0.2	g
Agar	15.0	g
Bromthymol Blue	0.08	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 24.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Allow to cool in a slanted position for use as slants. The agar also may be used as a plating medium.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Inoculate slants with growth from a pure culture using a light inoculum. Incubate all tubes for 4 days at 35 ± 2°C in an aerobic atmosphere.

Expected Results
A positive reaction is indicated by growth with an intense blue color in the slant. A negative reaction is evidenced by no growth to trace growth with no change in color (medium remains dark green).

Consult appropriate texts for additional differentiating characteristics.[4,5]

References
1. Koser. 1923. J. Bacteriol. 8:493.
2. Simmons. 1926. J. Infect. Dis. 39:209.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

User Quality Control
Identity Specifications
BBL™ Simmons Citrate Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain many dark and gray flecks.
Solution:	2.42% solution, soluble in purified water upon boiling. Solution is medium to dark, green, clear to slightly hazy.
Prepared Appearance:	Medium to dark, green, clear to slightly hazy, with a small amount of precipitate and as many as a large amount of insolubles.
Reaction of 2.42% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response
BBL™ Simmons Citrate Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 4 days.

ORGANISM	ATCC™	RECOVERY	REACTION
Enterobacter aerogenes	13048	Good	Alkaline (blue)
Escherichia coli	25922	Partial to complete inhibition	–
Klebsiella pneumoniae	33495	Good	Alkaline (blue)
Shigella flexneri	9199	Complete inhibition	–

Uninoculated Tube | *Enterobacter aerogenes* ATCC™ 13048 | *Escherichia coli* ATCC™ 25922

Availability

BBL™ Simmons Citrate Agar

AOAC BAM CCAM COMPF ISO

Cat. No. 211620 Dehydrated – 500 g
221026 Prepared Slants – Pkg. of 10*
221027 Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

Skim Milk • Skim Milk Medium

Intended Use

Skim Milk is used for preparing microbiological culture media. Skim Milk Medium may be used for the cultivation and differentiation of microorganisms based on the coagulation and proteolysis of casein.

Summary and Explanation

Skim Milk is soluble, spray-dried skim milk. When prepared in a 10% solution, it is equivalent to fresh skim milk. Skim Milk can be used to prepare Skim Milk Agar for detecting proteolytic microorganisms in foods,[1] including dairy products.[2] It can also be used to prepare litmus milk, a differential test medium for determining lactose fermentation and for detecting proteolytic enzymes that hydrolyze casein (milk protein) and cause coagulation (clot formation).[3]

Skim Milk Medium, 10% skim milk solution prepared in tubes, is used for the maintenance and propagation of lactic acid bacteria. It is especially useful in species differentiation within the genus *Clostridium*.

Principles of the Procedure

Skim Milk is a source of lactose and casein and other nutrients required for the growth of lactobacilli.[4] Clostridial species can be differentiated based on their ability to enzymatically degrade proteins to peptones (peptonization) or coagulate milk.[5] It may be used to detect the stormy fermentation produced by *Clostridium perfringens*.

User Quality Control

Identity Specifications

Difco™ Skim Milk

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	10% solution, soluble in purified water upon warming. Solution is white, opalescent.
Prepared Appearance:	Off-white to beige, opaque.
Reaction of 10% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Difco™ Skim Milk

Prepare the medium per label directions. Inoculate with a drop or loopful of fresh culture and incubate at 35 ± 2°C for 1-7 days.

ORGANISM	ATCC™	GROWTH	APPEARANCE
Clostridium perfringens	12919	Good	Stormy fermentation
Escherichia coli	25922	Good	Acid, curd
Lactobacillus rhamnosus	9595	Good	Acid, curd

Formula

Difco™ Skim Milk

Approximate Formula* Per Liter
Skim Milk Powder ..... 100.0 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 100 g of the powder in 1 L of purified water.
2. Warm, if necessary, to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Heat the medium in a boiling water bath for 2-5 minutes with caps loosened and cool to room temperature with caps tightened.

Inoculate tubes using a calibrated loop or sterile disposable pipet. For the study of anaerobic organisms, sterile mineral oil can be layered over the medium following inoculation.

Incubate tubes, with tightened caps for clostridia and loosened caps for other organisms, at 35 ± 2°C and read at intervals for 7 days for growth and reactions.

Expected Results

Consult an appropriate reference for the expected reactions for specific microbial species.[4,5]

Limitation of the Procedure

Skim Milk Medium supports growth of many microorganisms. Perform microscopic examination and other biochemical tests to identify isolates to the genus and species level, if necessary.

References

1. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, Washington, D.C.
2. Wehr and Frank (ed.). 2004. Standard methods for the examination of the dairy products, 17th ed. American Public Health Association, Washington, D.C.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Sneath and Holt (ed.). 1986. Bergey's Manual™ of systematic bacteriology, vol.2. Williams & Wilkins, Baltimore, Md.
5. Allen, Emery and Siders. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Skim Milk

Cat. No. 232100 Dehydrated – 500 g

BBL™ Skim Milk Medium

Cat. No. 298240 Prepared Tubes (D Tubes) – Pkg. of 10

Skirrows Medium

(See Campylobacter Agars)

Snyder Test Agar

Intended Use

Snyder Test Agar, also known as BCG Dextrose Agar,[1] is used for estimating the relative number of lactobacilli in saliva based on acid production.

Summary and Explanation

Tooth decay (dental caries) is a localized, progressive demineralization of the hard tissues of the crown and root surfaces of teeth. *Streptococcus mutans* and possibly lactobacilli ferment dietary carbohydrates that produce acids that cause the demineralization. The organisms reside in dental plaque, which is a gelatinous material that adheres to the surfaces of teeth. Demineralization of the tooth alternates with periods of remineralization. If demineralization exceeds remineralization, a subsurface carious lesion becomes a clinical cavity with extension of the decay into the dentine.[2]

Snyder[3,4] described a test procedure for determining, by colorimetric analysis, the rate and amount of acid produced by microorganisms in saliva. The procedure uses an agar medium that is known as Snyder Test Agar. Alban[5] simplified the procedure, used it extensively and reported it to be more accurate than Snyder's original procedure.

Principles of the Procedure

Snyder Test Agar contains peptones as sources of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate. Bromcresol green is the pH indicator. Agar is the solidifying agent.

Microorganisms that use the dextrose in the medium acidify the medium and the pH indicator, bromcresol green, changes color from blue-green to yellow.

Formula

Difco™ Snyder Test Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	10.0	g
Pancreatic Digest of Casein	10.0	g
Dextrose	20.0	g
Sodium Chloride	5.0	g
Agar	20.0	g
Bromcresol Green	0.02	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 65 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Specimens should be collected preferably before breakfast, lunch, or dinner, and before the teeth are brushed. This procedure can be done just before lunch or dinner.

User Quality Control

Identity Specifications

Difco™ Snyder Test Agar

Dehydrated Appearance:	Cream with green tint to light green, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is dark emerald green, very slightly to slightly opalescent.
Prepared Appearance:	Dark emerald green, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 4.8 ± 0.2

Cultural Response

Difco™ Snyder Test Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID PRODUCTION
Lactobacillus rhamnosus	9595	10^2-10^3	Good	+
Lactobacillus fermentum	9338	10^2-10^3	Good	+
Staphylococcus aureus	25923	10^2-10^3	None to poor	–

Uninoculated Tube

Lactobacillus fermentum ATCC™ 9338

Snyder Procedure[3,4]

1. Collect specimens of saliva in a sterile container while patient is chewing paraffin for 3 minutes.
2. Shake specimens thoroughly and transfer 0.2 mL to a tube of sterile Snyder Test Agar melted and cooled to 45°C. (Prepared medium in tubes is heated in a boiling water bath for 10 minutes and cooled to 45°C.)
3. Rotate the inoculated tubes to mix the inoculum uniformly with the medium and allow to solidify in an upright position.
4. Incubate at 35°C. Observe color at 24, 48 and 72 hours.

Alban Modification[5]

1. Collect enough unstimulated saliva to just cover the medium in the tube. When specimen collection is difficult, dip a sterile cotton swab into the saliva under the tongue or rub on tooth surfaces and place the swab just below the surface of the medium.
2. Incubate the inoculated tubes and an uninoculated control at 35°C.
3. Examine tubes daily for 4 days.
4. Observe daily color change compared to control tube.

Expected Results

Snyder Procedure

Observe tubes for a change in color of the medium from bluish-green (control) to yellow. A positive reaction is a change in color so that green is no longer dominant; record as ++ to ++++. A negative reaction is no change in color or only a slight change (green is still dominant); record as 0 to +.

Alban Modification

a. No color change
b. Color beginning to change to yellow from top of medium down (+)
c. One half of medium yellow (++)
d. Three fourths of medium yellow (+++)
e. The entire medium is yellow (++++)

The final report is a composite of the daily readings, for example; –, +, ++, +++. The readings indicate the rapidity and amount of acid production.

Limitations of the Procedure

1. The data indicate only what is happening at the time the specimen was collected.
2. At least two specimens collected within 2-4 days must be obtained to establish a base-line or reference point.
3. Only when two or more specimens have been cultured can any reliability or prediction be obtained.
4. The clinician must study enough cases by use of periodic laboratory data to establish the value or significance for the purpose intended.

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Lewis and Ismail. 1995. Can. Med. Assoc. J. *152*:836.
3. Snyder. 1941. J. Dent. Res. *20*:189.
4. Snyder. 1941. J. Am. Dent. Assoc. *28*:44.
5. Alban. 1970. J. Dent. Res. *49*:641.

Availability

Difco™ Snyder Test Agar

Cat. No. 224710 Dehydrated – 500 g

Sodium Hippurate Broth

Intended Use

Sodium Hippurate Broth is used in the determination of the ability of an organism to hydrolyze sodium hippurate by enzymatic action.

Summary and Explanation

Ayers and Rupp discovered that hemolytic streptococci from human and bovine sources could be differentiated by their ability to hydrolyze sodium hippurate.[1] Facklam et al. modified the test procedure in their study of the presumptive identification of groups A, B and D streptococci.[2]

The ability of an organism to hydrolyze sodium hippurate is one of a number of tests that aid in the differentiation of bovine beta-hemolytic group B *Streptococcus* (*S. agalactiae*) from human beta-hemolytic group B *Streptococcus* species.[3] Differentiation of beta-hemolytic group B streptococci from beta-hemolytic group A streptococci and nonenterococcal group D streptococci is also aided by the determination of hippurate hydrolysis.[3]

Principles of the Procedure

Heart muscle infusion and animal tissue peptone supply the variety of nutrients required for the growth of a majority of bacterial species. Sodium chloride maintains osmotic equilibrium. Sodium hippurate serves as a substrate for the measurement of hippurate hydrolysis.

Procedure

Inoculate tubes with one or two drops of an 18- to 24-hour pure broth culture of a confirmed beta-hemolytic *Streptococcus* or with one to two isolated colonies from an original isolation plate. Include an uninoculated tube as a negative control and a positive control (*S. agalactiae*). Incubate tubes with loosened caps for 48 hours at 35 ± 2°C in an aerobic atmosphere.

Following incubation, centrifuge all cloudy cultures and use the supernatant in the test. Aseptically transfer an aliquot of culture (or its supernatant) to small test tubes using 0.8 mL of the test organism cultures. Set up five negative controls. Add 0.8 mL from the incubated negative control tube to small negative control tube #1 and 1.0 mL aliquots to small negative control tubes #2 through #5.

Before adding ferric chloride solution to tubes containing test organism or positive cultures, use the small, negative control tubes to determine the amount of ferric chloride to add by means of the following procedure.

1. To small negative control tube #1 containing 0.8 mL of incubated broth, add 0.2 mL of the 12% ferric chloride solution and immediately shake gently.
2. Allow the tube to stand 10-15 minutes before reading the result.
3. If negative (initial precipitate clears within 15 minutes), the ferric ion is in excess and the ferric chloride solution can be used in the testing of the test cultures.
4. If positive (initial precipitate does not clear within 15 minutes), ferric ion is not in excess and must be titrated to determine the optimal amount of solution required to be in excess.

If titration is necessary, rapidly add 0.2, 0.3, 0.4 and 0.5 mL amounts of ferric chloride solution to small negative control tubes #2 through #5 each containing 1.0 mL of the incubated uninoculated broth. Immediately shake gently. Let stand 10-15 minutes with occasional shaking. The smallest amount of $FeCl_3$ solution giving a clear solution indicates that ferric ion is in excess. Use this amount in the evaluation of the test cultures.

Add the appropriate amount of the ferric chloride solution to all tubes containing 0.8 mL of incubated test and positive control cultures.

Expected Results

A positive test for hippurate hydrolysis is indicated by production of a brown flocculating, insoluble precipitate that persists on shaking. The amount of the precipitate is related to the degree of hippurate hydrolysis.

A negative test (hippurate not hydrolyzed) is indicated by the lack of precipitate formation or the formation of a precipitate that dissolves on shaking.

Consult an appropriate text for additional differentiating characteristics.[4]

References

1. Ayers and Rupp. 1922. J. Infect. Dis. *30*:388.
2. Facklam, Padula, Thacker, Wortham and Sconyers. 1974. Appl. Microbiol. 27:107.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Sodium Hippurate Broth

Cat. No. 221618 Prepared Tubes (K Tubes) – Pkg. of 10

Sorbitol MacConkey Agars

(See MacConkey Agars with Sorbitol)

Soybean-Casein Digest Media

*(See Tryptic/**Trypticase**™ Soy Agar and Tryptic/**Trypticase**™ Soy Broth)*

Soytone

*(See **Phytone**™ Peptone)*

Special Yeast and Mold Medium

Intended Use

Special Yeast and Mold Medium is used for isolating and cultivating yeasts and molds.

Summary and Explanation

In 1986, the American Congress of Governmental Industrial Hygienists (ACGIH) described a medium for detecting and enumerating fungi.[1] The ACGIH recommendations were accepted, with the addition of antibiotics, by the Finnish Medical Board which recommends this medium for collecting airborne microorganisms.[2]

Principles of the Procedure

Special Yeast and Mold Medium is a nutritionally rich medium that supports the growth of a wide variety of yeasts and molds. The medium contains malt extract, peptone and dextrose as sources of nutrients. Streptomycin and/or chlor-tetracycline may be added as antibacterial agents.[2]

Formula

Difco™ Special Yeast and Mold Medium

Approximate Formula* Per Liter		
Malt Extract	20.0	g
Peptone	1.0	g
Dextrose	20.0	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 61 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. For improved selectivity, aseptically add 40 mg streptomycin and/or 35 mg chlortetracycline and mix well.
5. If desired, adjust pH by aseptically adding 1N HCl or 1N NaOH.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates aerobically at room temperature in the dark for 4-7 days. Examine plates for growth after 18-24 hours incubation and up to 7 days.

Expected Results

Yeasts and molds should show growth in 4-7 days at room temperature. Bacteria should be inhibited on the complete medium containing antibacterial agents.

References

1. Morey, Otten, Burge, Chatigny, Feeley, LaForce and Peterson. 1986. Applied Industrial Hygiene *1*:R-19.
2. Guide regarding condition and attendance of houses. 1990. Finnish Medical Board. Helsinki, Finland.

Availability

Difco™ Special Yeast and Mold Medium

Cat. No. 210810 Dehydrated – 500 g

User Quality Control

Identity Specifications

Difco™ Special Yeast and Mold Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.1% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, very slightly to slightly opalescent.
Reaction of 6.1% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Difco™ Special Yeast and Mold Medium

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 7 days for the yeast and molds. Inoculate and incubate other organisms at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	26790	30-300	Good
Enterococcus faecalis	29212	30-300	Marked to complete inhibition
Escherichia coli	25922	30-300	Marked to complete inhibition
Trichophyton mentagrophytes	9533	30-300	Poor to fair

S

Spirit Blue Agar Lipase Reagent

Intended Use

Spirit Blue Agar is for use with Lipase Reagent or other lipid source for detecting and enumerating lipolytic microorganisms.

Summary and Explanation

In 1941, Starr[1] described a lipid emulsion medium for detecting lipolytic (lipase-producing) microorganisms to which he added the dye, spirit blue. Other dyes used as indicators of lipolysis were toxic to many microorganisms. Spirit blue did not have toxic effects. When testing samples of dairy products, air and sewage on Spirit Blue Agar, Starr obtained accurate counts of lipolytic microorganisms and total microbial counts on the same medium.

Lipolytic microorganisms, such as psychrotrophic bacteria, molds or yeasts, can adversely affect the flavor of milk and high fat dairy products. Spirit Blue Agar is a recommended medium for testing milk and dairy products.[2]

Lipase Reagent, a mixture of tributyrin and polysorbate 80, is recommended as the lipid source. Other lipoidal emulsions may be prepared from cottonseed meal, cream, vegetable oil and olive oil. A satisfactory emulsion can be prepared by dissolving 10 g gum acacia or 1 mL polysorbate 80 in 400 mL warm purified water, adding 100 mL cottonseed or olive oil and agitating vigorously to emulsify.

Principles of the Procedure

Spirit Blue Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Spirit blue is the indicator of lipolysis. Agar is the solidifying agent.

User Quality Control

Identity Specifications

Difco™ Spirit Blue Agar

Dehydrated Appearance:	Grayish-beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is royal blue, slightly opalescent.
Prepared Appearance:	Plain – Royal blue, opalescent.
	With 3% Lipase Reagent – Pale blue, opalescent.
Reaction of 3.5% Solution at 25°C:	pH 6.8 ± 0.2

Difco™ Lipase Reagent

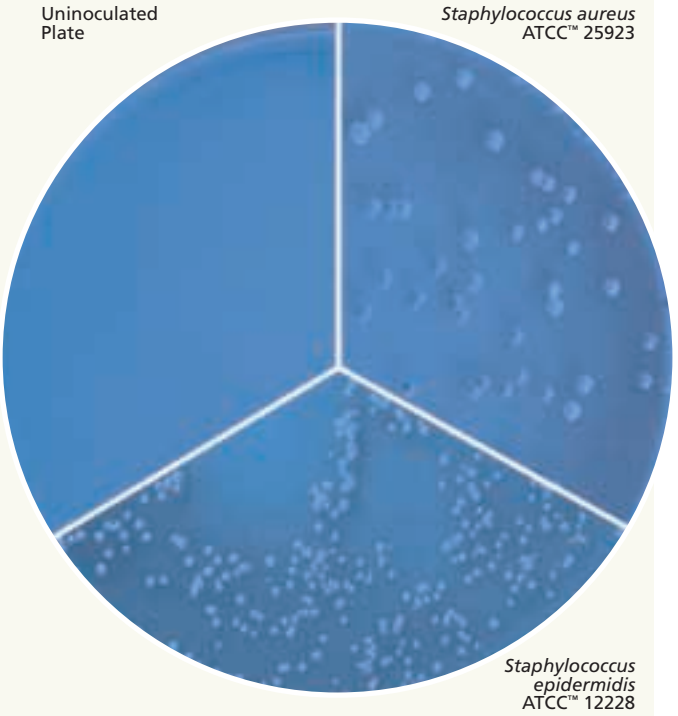
Appearance:	White, opaque emulsion.

Cultural Response

Difco™ Spirit Blue Agar and Lipase Reagent

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HALO/ LIPOLYSIS
Proteus mirabilis	25933	10^2-10^3	Good	–
Staphylococcus aureus	25923	10^2-10^3	Good	+
Staphylococcus aureus	6538	10^2-10^3	Good	+
Staphylococcus epidermidis	12228	10^2-10^3	Good	+



Lipase Reagent contains tributyrin, a true fat and the simplest triglyceride occurring in natural fats and oils. It is a good substrate when testing for lipolytic microorganisms because some microorganisms that hydrolyze tributyrin will not hydrolyze other triglycerides or fats containing longer chain fatty acids.[2]

Formulae

Difco™ Spirit Blue Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Yeast Extract	5.0	g
Agar	20.0	g
Spirit Blue	0.15	g

Difco™ Lipase Reagent

A ready-to-use lipid suspension, containing a mixture of tributyrin and polysorbate 80.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 35 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Aseptically add 30 mL Lipase Reagent or other lipid source and mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the organism onto the medium. Incubate plates at 35 ± 2°C for up to 72 hours, or at other temperatures and times according to standard methods.[2]

Expected Results

Lipolytic microorganisms metabolize the lipid in the medium and form colonies with halos indicating lipolysis.

References

1. Starr. 1941. Science 93:333.
2. Frank and Yousef. 2004. *In* Wehr and Frank (ed.), Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Spirit Blue Agar

SMD

Cat. No.	295010	Dehydrated – 100 g
	295020	Dehydrated – 500 g

Difco™ Lipase Reagent

SMD

Cat. No.	243110	Bottle – 6 × 20 mL

Spirolate Broth

Intended Use

Spirolate Broth was developed for mass culture of the Reiter treponeme in a medium without agar. It can be used for cultivating other spirochetes.

Summary and Explanation

In 1956, Omata and Disraely developed FM Medium as a selective medium for the growth of oral fusobacteria.[1] BBL™ Spirolate Broth is a modification of that medium and is recommended for bulk production of Reiter treponemes for use in antigen production or research studies. Supplementation with fatty acids has a stimulatory effect on growth of the Reiter treponeme.[2]

Principles of the Procedure

The casein peptone, dextrose and yeast extract supply nitrogenous growth factors, carbon, minerals and vitamins required for the metabolism of Reiter treponemes. Sodium chloride aids in the maintenance of the osmotic equilibrium of the medium. Sodium thioglycollate reduces the oxygen tension to a level conducive to the growth of treponemes. L-Cysteine hydrochloride is a reducing agent and is slightly inhibitory to fusobacteria.[1]

The addition of ether-soluble pure palmitic, stearic, oleic and linoleic acids, in equal amounts at a total fatty acid concentration of 0.20-0.25 mg/mL of medium, provides fatty acids which enhance the growth of Reiter treponemes.[2]

User Quality Control

Identity Specifications

BBL™ Spirolate Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.9% solution, soluble in purified water upon boiling. Solution is light to medium, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Light to medium, tan to yellow, clear to slightly hazy.
Reaction of 2.9% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

BBL™ Spirolate Broth

Prepare the medium per label directions (with 10% inactivated rabbit serum). Inoculate with a fresh culture and incubate at 35 ± 2°C for 7 days.

ORGANISM	RECOVERY
Reiter treponeme	Good

Formula

BBL™ Spirolate Broth

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Dextrose	5.0	g
Yeast Extract	5.0	g
Sodium Chloride	2.5	g
Sodium Thioglycollate	0.5	g
L-Cysteine HCl	1.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 29 g of the powder in 1 L of purified water. Mix thoroughly. (Add equal quantities of palmitic, stearic, oleic and linoleic acids to a total fatty acid concentration of 0.20-0.25 g/L, if desired.)
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in test tubes, filling them half full, using 15-20 mL in 6-inch tubes, preferably with screw caps. If larger containers are used, the ratio of surface to volume should be similar to that for tubes.
4. Autoclave at 121°C for 15 minutes. Close caps upon removal from the autoclave.
5. Cool and add sterile inactivated sheep, rabbit or bovine serum, 10% by volume, to each tube. Tighten caps.
6. Store at room temperature, not in the refrigerator, unless in sealed containers.
7. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate containers of Spirolate Broth with 0.05-mL aliquots of a 7-day pure culture in Thioglycollate Medium without Indicator-135C supplemented with 15% inactivated sheep, rabbit or bovine serum. Incubate containers for a minimum of 7 days at 35 ± 2°C in an anaerobic atmosphere (**BD GasPak™** EZ anaerobic system or equivalent).

Expected Results

After obtaining sufficient growth, process the cultures according to the particular method being utilized.

References

1. Omata and Disraely. 1956. J. Bacteriol. 72: 677.
2. Power and Pelczar. 1959. J. Bacteriol. 77: 789.

Availability

BBL™ Spirolate Broth

Cat. No. 211636 Dehydrated – 500 g

Standard Methods Agar

(See Plate Count Agar)

Standard Methods Agar with Lecithin and Polysorbate 80

Intended Use

Standard Methods Agar with Lecithin and Polysorbate 80 is recommended for the detection and enumeration of microorganisms present on surfaces of sanitary importance.

Summary and Explanation

Standard Methods Agar with the neutralizers, lecithin and polysorbate 80, is formulated according to recommendations of the American Public Health Association.[1,2] It is primarily used in **RODAC**™ (Replicate Organism Detection and Counting) and contact plates for the enumeration of microorganisms on flat impervious surfaces. For this purpose the plates must be prepared carefully to ensure the presence of a meniscus of agar extending above the top of the poured plate. This requires approximately 17.0 mL of sterile medium per **RODAC** or contact plate.

The presence and number of microorganisms on a surface is determined by the appearance of colonies on the surface of the medium following application to the test surface.[3] Collection of "samples" from identical areas before and after treatment with disinfectant yields data useful in evaluating cleaning procedures in environmental sanitation.

User Quality Control

Identity Specifications

BBL™ Standard Methods Agar with Lecithin and Polysorbate 80

Dehydrated Appearance:	Medium fine, softly lumped powder "brown sugar appearance," free of extraneous material.
Solution:	2.92% solution, soluble in purified water upon boiling. Solution is light, yellow to tan, slightly to moderately hazy.
Prepared Appearance:	Light, yellow to tan, slightly to moderately hazy.
Reaction of 2.92% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

BBL™ Standard Methods Agar with Lecithin and Polysorbate 80

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
Pseudomonas aeruginosa	10145	10^3-10^4	Good	Yellow to green pigment
Staphylococcus aureus	25923	10^3-10^4	Good	Cream to gold colonies

Principles of the Procedure

Casein peptone, yeast extract and dextrose are sources of nutrients required for the replication of microorganisms. The peptone provides nitrogenous compounds, including essential amino acids. Yeast extract is a rich source of B-complex vitamins. Dextrose is an energy source.

Lecithin and polysorbate 80, two commonly used neutralizers, are reported to inactivate residual disinfectants where the samples are being collected. Lecithin is incorporated to neutralize quaternary ammonium compounds, and polysorbate 80 is used to neutralize substituted phenolic disinfectants.[3-5]

Formula

BBL™ Standard Methods Agar with Lecithin and Polysorbate 80

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Yeast Extract	2.5	g
Dextrose	1.0	g
Agar	15.0	g
Lecithin	0.7	g
Polysorbate 80	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 29.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to approximately 45°C.
4. In **RODAC** or Contact plates, use 16.5-17.5 mL per plate.
5. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: The dehydrated medium has a characteristic "brown sugar" appearance and may seem moist.

Procedure

Liquefy the tubed medium in boiling water. Cool to 45-50°C and carefully pour in sterile **RODAC** plates. The agar in these plates after hardening should form a meniscus above the sides of the plates.

For use in the sampling of surfaces, remove the top of the plate. Apply the agar surface to a flat surface, pressing down gently but firmly and making certain that the entire agar meniscus touches the surface. Use a rolling uniform pressure on the back of the plate to effect contact. Lift the plate straight up from the surface, being careful not to allow it to slide along the surface. Replace the top of the plate. Incubate plates with the agar side up at 32°C for 24-48 hours depending upon whether contamination is heavy or light.[1,2]

Expected Results

After incubation, count the colonies and record as either number of colonies per **RODAC** plate or number of colonies per cm^2.[1,2] Subculture those colonies which are of interest so that positive identification can be made by means of biochemical testing and/or microscopic examination of organism smears.

References

1. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
3. McGowan. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
4. Quisno, Gibby and Foter. 1946. Am. J. Pharm. *118*: 320.
5. Erlandson and Lawrence. 1953. Science *118*: 274.

Availability

BBL™ Standard Methods Agar with Lecithin and Polysorbate 80

COMPF SMD

Cat. No.	211643	Dehydrated – 500 g*
	221939	Prepared Contact Plates – Pkg. of 20*
	221032	Prepared Pour Tubes, 18 mL – Pkg. of 10*

**Store at 2-8°C.*

m Staphylococcus Broth

Intended Use

m Staphylococcus Broth is used for isolating staphylococci by the membrane filtration technique.

Summary and Explanation

Staphylococci, along with other bacteria, are indicators of recreational water quality.[1] Indicators of health risk include normal skin flora that are likely to be shed, such as *Pseudomonas*, *Streptococcus* and *Staphylococcus*.[2] These organisms account for a large percentage of swimming pool-associated illness.[1]

The coagulase-positive species, *Staphylococcus aureus*, is well documented as a human opportunistic pathogen.[3] Coagulase-negative *Staphylococcus* spp. are a major component of the normal microflora of humans.[3] Staphylococci are widespread in nature, though they are mainly found living on the skin, skin glands and mucous membranes of mammals and birds.[3]

Chapman[4] added 7.5% NaCl to Phenol Red Mannitol Agar to achieve a selective medium for staphylococci. While studying this medium formulation, Chapman[5] developed Staphylococcus Medium 110. m Staphylococcus Broth is patterned after the formula of Staphylococcus Medium 110.

m Staphylococcus Broth, with the addition of sodium azide, is used in a multiple-tube procedure to monitor swimming pool water for the presence of *S. aureus*.[1]

Principles of the Procedure

Peptone provides the nitrogen, amino acids and minerals in m Staphylococcus Broth. Yeast extract is the vitamin source in this formula. Lactose and mannitol are the carbohydrates for bacterial growth. Dipotassium phosphate is the buffering agent. The high concentration of sodium chloride permits this medium to be selective for staphylococci.

Formula

Difco™ m Staphylococcus Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Yeast Extract	2.5	g
Lactose	2.0	g
Mannitol	10.0	g
Dipotassium Phosphate	5.0	g
Sodium Chloride	75.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 104 g of the powder in 1 L of purified water.
2. Warm slightly to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.

NOTE: For field studies where autoclaving is not practical, boil the medium for 5 minutes.

4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Follow the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.[1]
2. Use 2.0-2.5 mL of medium to saturate the paper pads on which the inoculated membrane is placed.
3. Incubate at 35 ± 2°C for 40-48 hours.

Expected Results

Observe membranes for growth and pigment production. Test for mannitol fermentation by adding a drop of bromthymol blue to the site from which a colony is removed; a yellow color indicates mannitol fermentation.

User Quality Control

Identity Specifications

Difco™ m Staphylococcus Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	10.4% solution, soluble in purified water upon warming. Solution is light amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 10.4% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ m Staphylococcus Broth

Prepare the medium per label directions. Use the membrane filtration technique with the test organisms. Inoculate and incubate at 35 ± 2°C under humid conditions for 40-48 hours. Observe the membranes for recovery and pigment production. Detect mannitol fermentation by adding a drop of bromthymol blue to the site where a colony was removed. A yellow color indicates a positive result for mannitol fermentation.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	MANNITOL FERMENTATION	PIGMENT PRODUCTION
Escherichia coli	25922	20-200	Inhibition	N/A	–
Staphylococcus aureus	25923	20-200	Good	+	+
Staphylococcus epidermidis	12228	20-200	Good	–	–

Limitation of the Procedure

Confirm positive isolates using biochemical reactions.

References

1. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
2. Seyfried, Tobin, Brown and Ness. 1985. Am. J. Public Health *75*:1071.
3. Kloos and Bannerman. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Chapman. 1945. J. Bacteriol. *50*:201.
5. Chapman. 1946. J. Bacteriol. *51*:409.

Availability

Difco™ m Staphylococcus Broth

SMWW

Cat. No. 264920 Dehydrated – 500 g

Staphylococcus Medium 110

Intended Use

Staphylococcus Medium 110, also known as Stone Gelatin Agar,[1] is used for isolating and differentiating staphylococci based on mannitol fermentation, pigment formation and gelatinase activity.

Summary and Explanation

Stone[2] described a culture medium on which food-poisoning staphylococci gave a positive gelatinase test. Chapman, Lieb and Curcio[3] later reported that pathogenic staphylococcal strains typically ferment mannitol, form pigment and produce gelatinase. Chapman[4] suggested adding 7.5% NaCl to Phenol Red Mannitol Agar to make a selective isolation medium for staphylococci using a high salt content. Further studies by Chapman[5] led to the development of Staphylococcus Medium 110. This medium is recommended for selectively isolating pathogenic staphylococci from foods.

Principles of the Procedure

Staphylococcus Medium 110 contains peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Sodium chloride, in high concentration, inhibits most bacteria other than staphylococci. Lactose and D-mannitol are the carbohydrates. Gelatin is included for testing liquefaction. Agar is the solidifying agent.

Pathogenic staphylococci (coagulase-positive staphylococci) typically resist the high salt concentration and form colonies with a yellow-orange pigment. These organisms typically ferment mannitol and produce acid, and liquefy gelatin, producing zones of clearing around the colonies.

Formula

Difco™ Staphylococcus Medium 110

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Yeast Extract	2.5	g
Gelatin	30.0	g
Lactose	2.0	g
D-Mannitol	10.0	g
Sodium Chloride	75.0	g
Dipotassium Phosphate	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Staphylococcus Medium 110

Dehydrated Appearance:	Very light beige to beige, free-flowing, homogeneous.
Solution:	14.9% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent to opalescent with heavy precipitate.
Prepared Appearance:	Light amber, slightly opalescent to opalescent.
Reaction of 14.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Staphylococcus Medium 110

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. To observe mannitol fermentation, remove a colony from the medium and add a drop of 0.04% bromthymol blue to the area from which the colony was removed. Observe for the formation of a yellow color (positive reaction).

To observe the gelatinase reaction, flood the plate with 5 mL of saturated ammonium sulfate solution and incubate at 35 ± 2°C for 10 minutes. Observe for a zone of clearing around the colonies (positive reaction).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	PIGMENT*	GELATINASE	MANNITOL
Escherichia coli	25922	10^2-3×10^2	Marked to complete inhibition	–	N/A	N/A
Staphylococcus aureus	25923	10^2-3×10^2	Good	+	+	+
Staphylococcus epidermidis	12228	10^2-3×10^2	Good	–	+	–

**Pigment is seen as a yellow to orange color.*

Directions for Preparation from Dehydrated Product

1. Suspend 149 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes.
4. Evenly disperse the precipitate when dispensing.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for procedures concerning selection and enumeration of staphylococci.

Expected Results

Growth of pathogenic staphylococci produces colonies with yellow-orange pigment.

Limitations of the Procedure

1. *Enterococcus faecalis* may grow on Staphylococcus Medium 110 as tiny colonies with mannitol fermentation. Differentiate these organisms from staphylococci with the Gram stain and catalase test.
2. Suspected staphylococci must be subcultured to Nutrient Broth, Blood Agar, BHI Broth, or Tryptose Phosphate Broth for coagulase testing as the high salt content of Staphylococcus Medium 110 may interfere with results.
3. Pigment production is not a reliable criterion for differentiation of staphylococcal species.

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Stone. 1935. Proc. Soc. Exp. Biol. Med. *33*:185.
3. Chapman, Lieb and Curcio. 1937. Food Res. *2*:349.
4. Chapman. 1945. J. Bacteriol. *50*:201.
5. Chapman. 1946. J. Bacteriol. *51*:409.

Availability

Difco™ Staphylococcus Medium 110

Cat. No. 229730 Dehydrated – 500 g

Japan

Cat. No. 251358 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Starch Agar

(For Nocardia, see Nocardia Differentiation Media)

Starch Agar

Intended Use
Starch Agar is used for cultivating microorganisms being tested for starch hydrolysis.

Summary and Explanation
In 1915,[1] Vedder formulated Starch Agar for cultivating *Neisseria*. Since then, other media have been developed that are superior to Starch Agar for the isolation of *Neisseria* spp., including enriched GC medium base. Starch Agar is used in differentiating microorganisms based on the starch hydrolysis test.

Principles of the Procedure
Beef extract provides the nitrogen, vitamins, carbon and amino acids in Starch Agar. Starch reacts with Gram Iodine to give a blue color. Organisms hydrolyzing starch through amylase production will produce a clearing around the isolate while the remaining medium is blue. Agar is the solidifying agent.

User Quality Control

Identity Specifications
Difco™ Starch Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.5% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 2.5% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response
Difco™ Starch Agar

Prepare the medium per label directions. Inoculate with a single streak of undiluted test organism and incubate at 35 ± 2°C for 40-48 hours. Test for starch hydrolysis by removing growth from each streak to expose the agar and flood plates with Gram Iodine.

ORGANISM	ATCC™	RECOVERY	STARCH HYDROLYSIS
Bacillus subtilis	6633	Good	+
Escherichia coli	25922	Good	–
Staphylococcus aureus	25923	Good	–
Streptococcus pyogenes	19615	Good	–

Formula
Difco™ Starch Agar

Approximate Formula* Per Liter

Beef Extract	3.0	g
Soluble Starch	10.0	g
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 25 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Starch Hydrolysis Test

Flood the surface of a 48-hour culture on Starch Agar with Gram Iodine.

For a complete discussion of the collection, isolation and identification of microorganisms, refer to appropriate references.[2,3]

Expected Results
Starch hydrolysis (+) is indicated by a colorless zone surrounding colonies. A blue or purple zone indicates that starch has not been hydrolyzed (-).

References
1. Vedder. 1915. J. Infect. Dis. *16*:385.
2. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability
Difco™ Starch Agar

Cat. No. 272100 Dehydrated – 500 g

Starch Agar with Bromcresol Purple

Intended Use
Starch Agar with Bromcresol Purple is a differential medium used for the primary isolation and presumptive identification of *Gardnerella vaginalis* from clinical specimens.

Summary and Explanation
Starch Agar with Bromcresol Purple was developed by Smith as a rapid means of detecting, isolating and enumerating *G. vaginalis* from specimens of the genitourinary tract.[1] Starch is useful as a differential agent because few organisms of the genitourinary tract other than *G. vaginalis* hydrolyze it.[2] The pH indicator, bromcresol purple, facilitates the detection of starch

hydrolysis by changing the color of the medium in the area of the reaction from purple to yellow.

Smith used Purple Broth Base as a basal medium. In this modification, GC II Agar base is used for improved recovery of *G. vaginalis* with soluble starch and bromcresol purple added for determining starch hydrolysis.

Principles of the Procedure

Enzymatic digests of casein and animal tissue provide amino acids and other nitrogenous substances. Corn starch neutralizes toxic fatty acids that may be present in the medium. The medium also contains sodium chloride to maintain the osmotic equilibrium and phosphate buffers to maintain the pH of the medium.

Hydrolysis of the starch produces acids, which lower the pH of the medium and change the color of the surrounding medium to yellow. The medium remains purple surrounding colonies of organisms that do not hydrolyze starch.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 35°C in a CO_2-enriched atmosphere for up to 72 hours.

Expected Results

After sufficient incubation, colonies of *G. vaginalis* and the medium in the area of growth become yellow, indicating acid production.

Gram staining, biochemical tests and other identification procedures should be performed to confirm findings.

References

1. Smith. 1975. Health Lab. Sci. *12*:219.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Starch Agar with Bromcresol Purple

Cat. No. 295911 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

S

Sterility Test Broth

(See Thioglycollate Media)

Stock Culture Agar

Intended Use

Stock Culture Agar is used for maintaining stock cultures of bacteria, particularly streptococci.

Summary and Explanation

Ayers and Johnson[1] reported a medium that gave luxuriant growth and extended viability of streptococci and other organisms. The success of their medium can be attributed to its semisolid consistency, added casein, buffered environment and dextrose, which serves as a readily available source of energy. This study reported that pathogenic streptococci remained viable for at least four months at room temperature (24°C) in the medium. Organisms such as *Streptococcus pneumoniae*, *Mycobacterium* spp. and others, grew well on their medium. Stock Culture Agar is prepared to duplicate the medium described by Ayers and Johnson.[1]

Stock Culture Agar may also be prepared with L-asparagine (1 g/L) for the maintenance of pathogenic and nonpathogenic bacteria, especially streptococci.[2]

Principles of the Procedure

Infusion from beef heart, peptone, gelatin and isoelectric casein provide the nitrogen, vitamins and amino acids in Stock Culture Agar. Dextrose is a carbon source. Disodium phosphate is a buffering agent. Sodium citrate acts as a preservative. Agar is the solidifying agent.

User Quality Control

Identity Specifications

Difco™ Stock Culture Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is medium amber, opalescent.
Prepared Appearance:	Medium amber, opalescent.
Reaction of 5% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Difco™ Stock Culture Agar

Prepare the medium per label directions. Stab inoculate with fresh cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY
Neisseria meningitidis	13090	Good
Staphylococcus aureus	25923	Good
Streptococcus pneumoniae	6305	Good
Streptococcus pyogenes	19615	Good

Formula

Difco™ Stock Culture Agar

Approximate Formula* Per Liter		
Beef Heart, Infusion from 500 g	10.0	g
Proteose Peptone	10.0	g
Gelatin	10.0	g
Isoelectric Casein	5.0	g
Dextrose	0.5	g
Disodium Phosphate	4.0	g
Sodium Citrate	3.0	g
Agar	7.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 50 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Ayers and Johnson. 1924. J. Bacteriol. 9:111.
2. Atlas. 1997. Handbook of microbiological media, 2nd ed. CRC Press, Inc., Boca Raton, Fla.

Availability

Difco™ Stock Culture Agar

Cat. No. 254100 Dehydrated – 500 g

Strep ID QUAD

Intended Use

The Streptococcus Identification Quadrant plate is a four-sectored plate that is used with a battery of tests for the differentiation and presumptive identification of streptococci.

Summary and Explanation

Quadrant I contains **Trypticase**™ Soy Agar, Modified (TSA II) supplemented with the antibiotic bacitracin. TSA II is a nutritious, general-purpose medium that provides excellent growth of a wide variety of microorganisms, including streptococci. TSA II with bacitracin is used for the presumptive differentiation of group A streptococci (*S. pyogenes*) from non-group A streptococci based on susceptibility to bacitracin.

Quadrant II contains TSA II enriched with sheep blood to provide appropriate hemolytic reactions and the erythrocytes needed for performing the CAMP test. The CAMP test enables the presumptive identification of group B streptococci.[1]

Quadrant III contains Bile Esculin Agar for the rapid selective detection and enumeration of group D streptococci. Rochaix initially noted the value of esculin hydrolysis in the identification of enterococci.[2] The enterococci are able to hydrolyze esculin while other streptococci cannot. Meyer and Schonfeld incorporated bile into the esculin medium and showed that 61 of 62 enterococci were able to grow and split esculin, whereas other streptococci could not.[3] Swan used a bile esculin medium containing 40% bile and reported that a positive reaction on this medium correlated with a serological group D precipitin reaction.[4] Facklam and Moody evaluated Swan's medium and concluded that the bile-esculin test provided a reliable means of identifying and differentiating group D streptococci.[5]

Quadrant IV contains Blood Agar Base with 6.5% sodium chloride for the determination of salt tolerance. Enterococcal streptococci may be easily differentiated from other streptococci by their ability to grow in the presence of salt.[6]

Principles of the Procedure

Quadrants I and II contain TSA II as a basal medium. TSA II contains digests of casein and soybean meal to supply amino acids and other complex nitrogenous substances.

The polypeptide antibiotic bacitracin is incorporated in quadrant I for presumptive differentiation of group A from non-group A beta-hemolytic streptococci. Group A beta-hemolytic streptococci are inhibited by the bacitracin while non-group A beta-hemolytic streptococci are resistant.

Defibrinated sheep blood is incorporated in quadrant II to enable the detection of hemolytic reactions. Defibrinated sheep blood is the most widely-used and efficient blood for enriching agar-based media. Hemolytic reactions are proper, and *Haemophilus haemolyticus*, a bacterium commonly found in nose and throat specimens that is indistinguishable from beta-hemolytic streptococci, is inhibited.[1]

The CAMP test is based on the formation of a zone of synergistic hemolysis at the junction of perpendicular streak inocula of *Staphylococcus aureus* and group B streptococci. The reaction is caused by the sphingomyelinase (beta-toxin) of *S. aureus* reacting with sphingomyelin in the sheep erythrocyte membrane to produce ceramide. A non-enzymatic protein (CAMP protein), produced by group B streptococci (*S. agalactiae*), binds to the ceramide and leads to disorganization of the lipid bilayer of the sheep erythrocyte membrane, resulting in an area of complete lysis that is shaped like an arrowhead or crescent.[1,7]

Bile Esculin Agar enables the differentiation of group D from non-group D streptococci. The bile in the esculin medium in quadrant III inhibits most gram-positive organisms other than group D streptococci. Esculin is incorporated as a differential agent. Hydrolysis of esculin by group D species results in the production of esculetin and dextrose. The esculetin reacts with the iron salt in the medium, producing a dark brown to black complex that appears as zones around the group D colonies.

Enterococcal species may be differentiated in quadrant IV by the 6.5% NaCl tolerance test. Enterococci usually grow heavily; nonenterococcal species do not grow in the salt-supplemented medium. However, salt tolerant, nonenterococcal streptococci do grow occasionally.[1]

Procedure

Subculture the organism to be tested onto a plate of **Trypticase** Soy Agar enriched with sheep blood, or another suitable medium, streaking to obtain isolated colonies. Incubate for 18-24 hours in a CO_2-enriched atmosphere. Using a sterile inoculating loop, choose one or two isolated colonies and perform a Gram stain, examining to confirm that the morphology of the isolate is appropriate for streptococci.

Choose three or four well-isolated colonies and streak the surface of quadrants I, III, and IV of the Strep ID QUAD plate.

Inoculate quadrant II by streaking *Staphylococcus aureus* ATCC™ 33862 across the widest area of the quadrant. If a loop is used, do not use it parallel to the agar surface, since the streak will be too wide and results will not be satisfactory. Then, streak the unknown isolate perpendicular to the *S. aureus* culture, leaving 2-3 mm space between the two streak lines. Alongside the unknown isolate, streak a known *S. agalactiae* strain as a positive control and *S. pyogenes* as a negative control. This procedure should be practiced with known cultures before using it to identify unknown isolates.

Incubate the plates in an inverted position (agar side up) in an aerobic atmosphere at 35 ± 2°C for 18-24 hours. Do not incubate anaerobically or in a CO_2 incubator. False-positive CAMP test results may occur with group A streptococci when incubation is in an anaerobic or CO_2-enriched atmosphere.[1,8]

Expected Results

Interpret results in conjunction with hemolytic reactions of the isolate.

Quadrant I (bacitracin): Streptococci strains susceptible to bacitracin are inhibited on quadrant I while bacitracin-resistant strains exhibit growth. A bacitracin-susceptible strain may be presumptively identified as a group A streptococcus.

Quadrant II (CAMP test): A definite arrowhead or crescent-shaped clearing at the junction of *S. aureus* and the isolate indicates a positive reaction. The absence of clearing indicates a non-group B streptococcus. Bacitracin-resistant, CAMP-positive, beta-hemolytic streptococci may be identified presumptively as group B streptococci. CAMP-positive group A streptococci may be differentiated from group B streptococci by hemolysis, bacitracin susceptibility and hippurate hydrolysis. Group B streptococci generally have smaller hemolytic zones than group A streptococci.

Quadrant III (Bile Esculin Agar): The presence of brownish-black to black pigmentation of the medium indicates that the isolate may be presumptively identified as a group D streptococcus. Any blackening of the medium is enough to report as a positive esculin reaction. The test is negative if there is no blackening of the medium.

Quadrant IV (6.5% sodium chloride): Growth indicates a positive reaction. No growth indicates a negative reaction. A bile esculin positive, salt-tolerant isolate may be presumptively identified as an enterococcus.

References

1. Ruoff. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. Rochaix. 1924. Comt. Rend. Soc. Biol. *90*:771.
3. Meyer and Schonfeld. 1926. Zentralbl. Bakteriol. Parasitenk. Infectionskr. Hyg. Abt. Orig. *99*:402.
4. Swan. 1954. J. Clin. Pathol. *7*:160.
5. Facklam and Moody. 1970. Appl. Microbiol. *20*:245.
6. Facklam, Padula, Thacker, Wortham and Sconyers. 1974. Appl. Microbiol. *27*:107.
7. Bernheimer, Linder and Avigard. 1979. Infect. Immun. *23*:838.
8. Darling. 1975. J. Clin. Microbiol. *1*:171.

Availability

BBL™ Strep ID QUAD

Cat. No. 297891 Prepared Plates (QUAD) – Pkg. of 10*

**Store at 2-8°C.*

Sulfite Agar

Intended Use

Sulfite Agar is used for detecting thermophilic, H_2S-producing anaerobes, particularly in foods.

Summary and Explanation

Sulfide spoilage of foods is due to three factors: high spore counts, the heat resistance of the spores and subjecting the finished product to elevated temperatures. The last factor may occur if the processed food is not cooled adequately.[1]

Clark and Tanner[2] described the thermophilic organisms that cause spoilage in canned foods as flat-sour spoilage organisms, thermophilic anaerobes and sulfide-spoilage organisms. They used Sulfite Agar to study sulfide-spoilage organisms in sugar and starch.

Both beet and cane sugar can carry spores of the thermophilic bacteria that are spoilage agents.[3] *Desulfotomaculum nigrificans*, first classified as *Clostridium nigrificans,* causes spoilage in non-acid canned foods such as vegetables and

User Quality Control

Identity Specifications

Difco™ Sulfite Agar

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Light amber, very slightly to slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Difco™ Sulfite Agar

Prepare the medium per label directions. Inoculate molten medium, solidify and incubate aerobically at 55 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	SULFITE REDUCTION
Bacillus stearothermophilus	10149	30-100	Good	–
Clostridium thermosaccharolyticum	7956	30-100	Good	+
Desulfotomaculum nigrificans	19858	30-100	Good	+

Uninoculated Tube | *Bacillus stearothermophilus* ATCC™ 10149 | *Desulfotomaculum nigrificans* ATCC™ 19858

infant formula.[1] The growth of *D. nigrificans* occurs in the range of pH 6.2-7.8, with the best growth occurring at pH 6.8-7.3. Scanty growth can be observed at pH 5.6. The reaction of most vegetables, except corn and peas, falls below pH 5.8, so sulfide spoilage is rare.[1]

Sulfite Agar is a recommended standard methods medium for detecting sulfide spoilage bacteria.[1,3]

Principles of the Procedure

Sulfite Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium sulfite, upon reduction, produces hydrogen sulfide. Agar is the solidifying agent.

Iron nails or iron strips will combine with any dissolved oxygen in the medium and provide an anaerobic environment.

Formula

Difco™ Sulfite Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Sodium Sulfite	1.0	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure[1]

Sugar

1. Place 20 g of dry sugar in a dry, sterile, graduated 250 mL Erlenmeyer flask closed with a rubber stopper.
2. Add sterile water to the 100 mL mark and shake to dissolve.
3. Replace the stopper with a sterile cotton plug, bring the solution rapidly to a boil, and continue boiling for 5 minutes.
4. Replace evaporated liquid with sterile water.
5. Cool immediately in cold water.

NOTE: For liquid sugar, prepare as for dry sugar, except determine the amount of liquid sugar needed on the basis of degree Brix in order to be equivalent to 20 g of dry sugar.[3]

6. Divide 20 mL of heated sugar solution among 6 screw-cap tubes (20 × 150 mm) containing approximately 10 mL of freshly autoclaved, still molten Sulfite Agar and a nail.
7. Make the inoculations into freshly autoclaved medium, and cool and solidify immediately in cold water.
8. Preheat the tubes to 50-55°C.
9. Incubate at 50-55°C for 24-48 hours.

Starch and Flour

1. Place 20 g of starch or flour in a dry, sterile, graduated 250 mL Erlenmeyer flask.
2. Add sterile water to the 100 mL mark, swirling occasionally.
3. Close the flask with a sterile rubber stopper.
4. Shake well to obtain a uniform, lump-free suspension. Add sterile glass beads to the sample mixture to aid in thoroughly mixing during shaking.

5. Divide 20 mL of the starch or flour suspension among 6 screw-cap tubes (20 × 150 mm) containing approximately 10 mL of freshly autoclaved, still molten Sulfite Agar and a nail.
6. Swirl the tubes several times to ensure even dispersion of the starch or flour in the medium. Heat in a boiling water bath for 15 minutes, continuing to swirl the tubes.
7. Cool and solidify immediately in cold water.
8. Preheat the tubes to 50-55°C.
9. Incubate at 50-55°C for 24-48 hours.

Nonfat Dry Milk

1. Place 10 g of nonfat dry milk in a sterile, graduated 250 mL Erlenmeyer flask.
2. Add .02N sodium hydroxide to the 100 mL mark.
3. Shake to completely dissolve.
4. Autoclave at 5 pounds pressure for 10 minutes.
5. Cool immediately.
6. Transfer 2 mL of nonfat dry milk solution to each of two screw-cap tubes (20 × 150 mm) containing freshly autoclaved, still molten Sulfite Agar and a nail.
7. Gently swirl several times.
8. Cool and solidify immediately in cold water.
9. Preheat the tubes to 50-55°C.
10. Incubate at 50-55°C for 24-48 ± 3 hours.

Cream

1. Mix 2 g of gum tragacanth and 1 g of gum arabic in 100 mL of water in an Erlenmeyer flask.
2. Autoclave at 121°C for 20 minutes.
3. Transfer 20 mL of cream sample to a sterile, graduated 250 mL Erlenmeyer flask.
4. Add sterilized gum mixture to the 100 mL mark.
5. Shake carefully using a sterile rubber stopper.
6. Loosen the stopper. Autoclave at 5 pounds pressure for 5 minutes.

Soy Protein Isolates

1. Prepare a 10% suspension of soy protein isolate in sterile 0.1% peptone water in milk dilution or similar bottles.
2. Adjust to pH 7.0 ± 0.1.
3. Steam in an autoclave at 5 pounds pressure for 20 minutes.
4. Add 1 mL of soy protein isolate suspension to each of 10 tubes containing freshly autoclaved, still molten Sulfite Agar and a nail. If using already prepared medium, heat the tubes immediately before inoculation to eliminate oxygen.
5. Mix tubes.
6. Solidify in an ice water bath.
7. Overlay with vaspar (one part mineral oil combined with two parts petroleum jelly, heated in an oven at 191°C for 3 hours).
8. Preheat the tubes to 55°C.
9. Incubate at 55°C for 14 days. Take preliminary counts at 48 hours, 7 days and 14 days in case tubes become completely blackened.
10. Count the blackened areas for each tube and report as the number of spores per gram of soy isolate.

Expected Results

Hydrogen sulfide production from the reduction of sulfite causes a blackening of the medium.

Sulfide spoilage spores should be present in not more than two of five samples tested (40%) with not more than 5 spores per 10 g in any one sample.[1]

Limitations of the Procedure

1. Nails or iron strips should be cleaned in hydrochloric acid and rinsed well to remove any rust before being placed into tubes of medium.
2. If iron nails or iron strips are not available, substitute 10 mL of 5% ferric citrate solution.
3. Spoiled peas may not show discoloration but will show blackening with a dark-colored brine.
4. Spangling of the enamel may occur as a result of the interaction of dissolved hydrogen sulfide with the iron of the container.

References

1. Donnelly and Hannah. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. Clark and Tanner. 1937. Food Res. 2:27.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Sulfite Agar

AOAC COMPF

Cat. No. 297210 Dehydrated – 500 g

Super Broth (Animal Free)

Intended Use

Select APS™ Super Broth is a molecular genetics medium used to grow *Escherichia coli* to a high cell density.

Summary and Explanation

The Select Alternative Protein Source (APS) media were designed as alternatives to classical animal-based media for the maintenance and propagation of *Escherichia coli* strains in molecular genetics procedures. **Select APS** media are manufactured from animal-free ingredients in order to minimize the risk of bovine spongiform encephalopathy in culture media containing animal, and especially bovine, materials.

Select APS Super Broth is based on the Terrific Broth formulation designed by Tartof and Hobbs.[1] The medium was developed for ease of use to improve yield of plasmid-bearing *E. coli* strains over that of LB Broth. The 1.2% tryptone in Terrific Broth was replaced with the animal-free component, soy hydrolysate, in the same concentration in **Select APS** Super Broth. The 2.4% yeast extract and recommended addition of 5 mL/L glycerol is the same for **Select APS** Super Broth as is used in classical Terrific Broth. The buffering system, 1.14% dipotassium phosphate and 0.17% monopotassium phosphate, is altered from that of classical Terrific Broth. The formulation for **Select APS** Super Broth does not contain glucose, thus preventing acetate build-up in the fermentation process.[2]

Select APS Super Broth was used in the production of purified recombinant human uteroglobin for the treatment of inflammatory and fibrotic conditions.[3] In addition, the medium was used in the process development and analysis of a preerythrocyte-stage protein-based vaccine for *Plasmodium falciparum*.[4] **Select APS** Super Broth was also used in a study to show that heterologous protein expression was enhanced by harmonizing the codon usage frequencies of the target gene with those of the expression host.[5]

User Quality Control

Identity Specifications

Difco™ Select APS™ Super Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.9% solution, soluble in purified water. Solution is medium to dark, yellow to tan, clear to moderately hazy.
Prepared Appearance:	Medium to dark, yellow to tan, clear to moderately hazy.
Reaction of 4.9% Solution at 25°C:	pH 6.8 – 7.5

Cultural Response

Difco™ Select APS™ Super Broth

Prepare the medium per label directions. Inoculate and incubate at 35-37°C, 250 rpm for 12 hours.

ORGANISM	ATCC™	RECOVERY
Escherichia coli	700790	Good

Principles of the Procedure

Soy hydrolysate provides nitrogen and carbon compounds for bacterial metabolism. Yeast extract supplies vitamins, amino acids and trace elements which enhance bacterial growth and plasmid yield. The phosphate buffering system prevents cell death caused by pH drop. Glycerol is added as a carbon and energy source which, unlike glucose, is not fermented to acetic acid.

Formula

Difco™ Select APS™ Super Broth

Approximate Formula* Per Liter		
Soy Hydrolysate	12.0	g
Yeast Extract	24.0	g
Dipotassium Phosphate	11.4	g
Monopotassium Phosphate	1.7	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 49.1 g of the powder and 5 mL of glycerol in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[6,7]

Expected Results

Growth is evident by the appearance of turbidity.

References

1. Tartof and Hobbs. 1987. Bethesda Research Laboratories Focus. 9:12.
2. Swartz. 2001. Curr. Opinion Biotechnol. 12:195.
3. World Intellectual Property Organization. 2003. WO/2003/003979 (Claragen, Inc.) 2003-1-16. World Intellectual Property Organization, Geneva, Switzerland.
4. Hillier, Ware, Barbosa, Angov, Lyon, Heppner and Lanar. 2005. Infect. Immun. 73:2109.
5. Angov, Hillier, Kincaid and Lyon. 2008. PLoS ONE. 3(5):e2189. Doi: 10.1371/ journal. pone.0002189.
6. Sambrook, and Russell. 2001. Molecular cloning, a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
7. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl. 2002. Short protocols in molecular biology, 5th ed. John Wiley & Sons, Inc., Hoboken, N.J.

Availability

Difco™ Select APS™ Super Broth

Cat. No.	212485	Dehydrated – 500 g
	212486	Dehydrated – 10 kg

Synthetic Broth AOAC

Intended Use
Synthetic Broth AOAC is used for maintaining disinfectant test cultures.

Summary and Explanation
Synthetic Broth AOAC is a chemically defined broth recommended by AOAC International (AOAC).[1] It contains all the nutrients essential for growth of the test cultures used in determining the phenol coefficients of disinfectants.

Principles of the Procedure
The chemically-defined ingredients in Synthetic Broth AOAC provide nitrogen, carbon, vitamins and minerals required for bacterial growth.

Formula
Difco™ Synthetic Broth AOAC

Approximate Formula* Per Liter		
L-Cystine	0.05	g
DL-Methionine	0.37	g
L-Arginine Hydrochloride	0.4	g
DL-Histidine Hydrochloride	0.3	g
L-Lysine Hydrochloride	0.85	g
L-Tyrosine	0.21	g
DL-Threonine	0.5	g
DL-Valine	1.0	g
L-Leucine	0.8	g
DL-Isoleucine	0.44	g
Glycine	0.06	g
DL-Serine	0.61	g
DL-Alanine	0.43	g
L-Glutamic Acid Hydrochloride	1.3	g
L-Aspartic Acid	0.45	g
DL-Phenylalanine	0.26	g
DL-Tryptophan	0.05	g
L-Proline	0.05	g
Sodium Chloride	3.0	g
Potassium Chloride	0.2	g
Magnesium Sulfate (anhydrous)	0.05	g
Monopotassium Phosphate	1.5	g
Disodium Phosphate	4.0	g
Thiamine Hydrochloride	0.01	g
Nicotinamide	0.01	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 17 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1-2 minutes to completely dissolve the powder.
3. Dispense 10 mL amounts into 20 × 150 mm culture tubes.
4. Autoclave at 121°C for 20 minutes.
5. Before inoculating, aseptically add 0.1 mL sterile 10% dextrose solution per tube.
6. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control
Identity Specifications
Difco™ Synthetic Broth AOAC

Dehydrated Appearance:	White, homogeneous, free-flowing.
Solution:	1.7% solution, soluble in purified water upon boiling. Solution is colorless and clear.
Prepared Appearance:	Colorless, clear.
Reaction of 1.7% Solution at 25°C:	pH 7.1 ± 0.1

Cultural Response
Difco™ Synthetic Broth AOAC

Prepare the medium per label directions (with 0.1 mL 10% dextrose added per tube). Inoculate and incubate the tubes at 35 ± 2°C for 48 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Salmonella enterica subsp. *enterica* serotype Choleraesuis	10708	~10^2	Good

Procedure
See the reference for specific procedures.[1]

Expected Results
Refer to the reference and procedures for results.[1]

Reference
1. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability
Difco™ Synthetic Broth AOAC

AOAC

Cat. No. 235220 Dehydrated – 500 g

TAT Broth Base • TAT Broth

Intended Use

TAT Broth Base with added polysorbate 20 and TAT Broth (complete) are used for cultivating microorganisms from highly viscous or gelatinous materials.

Summary and Explanation

TAT (Tryptone-Azolectin-Tween™*) Broth Base with the addition of polysorbate 20 is recommended for testing for the presence of microorganisms in viscous materials, such as salves or ointments. It is especially adapted to the testing of cosmetics. Cosmetics and pharmaceutical products are subject to contamination during manufacturing and use by consumers.[1] Preservatives are used in aqueous products to make them self-sterilizing for vegetative bacteria, yeasts and molds, and bacteriostatic or bactericidal for spores.[1] TAT Broth is the medium, including polysorbate 20, provided in bottles.

**Tween is a trademark of ICI Americas, Inc.*

User Quality Control

Identity Specifications

Difco™ TAT Broth Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.5% solution with 4% polysorbate 20. Soluble when heated to 50-60°C; let stand for 15-30 minutes with occasional agitation to dissolve prior to autoclaving. Solution is light amber, clear to very slightly opalescent, may have a very slight precipitate.
Prepared Appearance:	Light amber, clear to very slightly opalescent, with a very sight precipitate.
Reaction of 2.5% Solution w/4% Polysorbate 20 at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ TAT Broth

Prepare the medium per label directions or use prepared TAT Broth. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10^2-3×10^2	Good
Candida albicans	26790	10^2-3×10^2	Fair to good
Pseudomonas aeruginosa	27853	10^2-3×10^2	Good
Salmonella enterica subsp. *enterica* serotype Typhi	6539	10^2-3×10^2	Good
Staphylococcus aureus	25923	10^2-3×10^2	Good

Principles of the Procedure

Peptone provides the nitrogen, vitamins, amino acids and carbon in TAT Broth Base. Soy lecithin and polysorbate 20 neutralize preservatives in the cosmetics or pharmaceutical products, allowing bacteria to grow.

Formulae

Difco™ TAT Broth Base

Approximate Formula* Per 960 mL

Pancreatic Digest of Casein	20.0	g
Soy Lecithin	5.0	g

Difco™ TAT Broth (prepared)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0	g
Soy Lecithin	5.0	g
Polysorbate 20	40.0	mL

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 25 g of the powder in 960 mL of purified water.
2. Add 40 mL of polysorbate 20.
3. Heat in a water bath at 50-60°C for 15-30 minutes with occasional agitation to dissolve completely.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Add 10 g or 10 mL of an undiluted sample to 90 mL of complete medium and agitate to obtain an even suspension.
2. Incubate at 35 ± 2°C for 18-48 hours.

Expected Results

Tubes or bottles exhibiting growth should be subcultured for identification.

Reference

1. Orth. 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, N.Y.

Availability

Difco™ TAT Broth Base

Cat. No.	298410	Dehydrated – 500 g
	292848	Dehydrated – 2 kg

Difco™ TAT Broth

Cat. No.	290721	Prepared Bottles (wide mouth), 90 mL – Pkg. of 10

Bacto™ TC Lactalbumin Hydrolysate

Intended Use

Bacto TC Lactalbumin Hydrolysate is used for preparing bacterial, insect and mammalian cell culture media.

Summary and Explanation

Bacto TC Lactalbumin Hydrolysate is intended as a nutritional supplement for bacterial, insect and mammalian cell culture. For years, TC Lactalbumin Hydrolysate has been used as a nutritional source for lactobacilli. It is also useful for indole testing because of its high tryptophan content. TC Lactalbumin Hydrolysate is frequently used in mammalian cell culture media as an amino acid supplement.[1]

User Quality Control

Identity Specifications

Bacto™ TC Lactalbumin Hydrolysate

Dehydrated Appearance:	Buff to tan, free-flowing, homogeneous powder.
Solution:	1.0% solution, soluble in purified water. Solution is very light to light amber, clear.

Cultural Response

Bacto™ TC Lactalbumin Hydrolysate

Prepare a sterile solution containing TC Hanks solution with 0.5% **Bacto** TC Lactalbumin Hydrolysate and 10% fetal calf serum in tissue culture flasks. Prepare a second sterile solution containing TC Medium 199 with 0.5% **Bacto** TC Lactalbumin Hydrolysate and 2.5% fetal calf serum in tissue culture flasks. Inoculate flasks with epithelial and fibroblast cell lines. Assay cell growth is comparable to approved control after 7 days of incubation at 35 ± 2°C.

Principles of the Procedure

Bacto TC Lactalbumin Hydrolysate is the enzymatically hydrolyzed protein portion of milk whey. In recent years, whey proteins have gained increased recognition as a complete protein source.[2] This product is a mixture of peptides, amino acids, carbohydrates, simple and complex.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** TC Lactalbumin Hydrolysate in the formula of the medium being prepared. Add product as required.

Procedure

See appropriate references for specific procedures using **Bacto** TC Lactalbumin Hydrolysate.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Bridson and Brecker. 1970. Methods in Microbiology *3A*:248.
2. Burrington. 2002. The Dairy Pipeline. *14*:1.

Availability

Bacto™ TC Lactalbumin Hydrolysate

Cat. No.	259962	Dehydrated – 500 g
	259961	Dehydrated – 10 kg

T

Bacto™ TC Yeastolate • TC Yeastolate, UF

Intended Use

Bacto TC Yeastolate and TC Yeastolate, UF (ultra-filtered) are used for preparing bacterial, insect and mammalian cell culture media.

Summary and Explanation

Both **Bacto** TC Yeastolate and TC Yeastolate, UF are intended as nutritional supplements for bacterial, insect and mammalian cell culture. For years, TC Yeastolate has been used in insect cell nutrition. TC Yeastolate was found to be a very versatile supplement to enhance growth and production characteristics of Sf9 and High-Five cells.[1-5]

Principles of the Procedure

Bacto TC Yeastolate and TC Yeastolate, UF are animal-free, water-soluble portions of autolyzed *Saccharomyces cerevisiae*. Both products are a mixture of peptides, amino acids, carbohydrates, simple and complex, as well as vitamins. TC Yeastolate, UF has been ultra-filtered at a 10,000 MWCO (molecular weight cut-off). It has an endotoxin value of less than 500 EU/g.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** TC Yeastolate or TC Yeastolate, UF in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for specific procedures using **Bacto** TC Yeastolate or TC Yeastolate, UF.

User Quality Control

Identity Specifications

Bacto™ TC Yeastolate

Dehydrated Appearance:	Beige, free-flowing, homogeneous fine powder.
Solution:	0.1% and 0.2% solutions, soluble in TC Medium 199. Solutions, after the addition of 10% sodium bicarbonate to adjust the pH to 7.0-7.2, are orange-red, clear.

Difco™ TC Yeastolate, UF

Dehydrated Appearance:	Beige, free-flowing, homogeneous fine powder.
Solution:	2% solution, soluble in purified water. Solution is light to dark, yellow to tan, clear to slightly hazy.
Reaction of 2% Solution at 25°C:	pH 5.4-7.2

Expected Results

Refer to appropriate references and procedures for results.

References

1. Chan, Greenfield and Reid. 1998. Biotechnol. Bioeng. *59*:178.
2. Nguyen, Jarnagin, Williams, Chan and Barnett. 1993. J. Biotechnol. *31*:205.
3. Ikonomou, Bastin, Schneider and Agathos. 2001. In Vitro Cell Dev. Biol. Anim. *37*:549.
4. Bedard, Kamen, Tom and Maassie. 1994. Cytotechnology *15*:129.
5. Donalson and Shuler. 1998. Biotechnology Prog. *14*:573.

Availability

Bacto™ TC Yeastolate

Cat. No.	255772	Dehydrated – 100 g
	255771	Dehydrated – 10 kg

Difco™ TC Yeastolate, UF

Cat. No.	292804	Dehydrated – 500 g
	292805	Dehydrated – 10 kg

TCBS Agar

Intended Use

Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS Agar) is used for the selective isolation of cholera vibrios and *Vibrio parahaemolyticus* from a variety of clinical and nonclinical specimens.[1,2]

Summary and Explanation

Vibrio species are most widely recognized for their role in human intestinal infections. Diarrheas caused by *Vibro cholerae* and *V. parahaemolyticus* are important worldwide.[3] The isolation of *Vibrio* species has been enhanced by the development of media which are highly selective for vibrios.

TCBS is the primary plating medium universally used for the selective isolation of vibrios that cause cholera, diarrhea and food poisoning. It was developed by Kobayashi et al.[4], who modified the selective medium of Nakanishi.[5] The combination of alkaline peptone water and TCBS Agar is used in many procedures for the isolation of *V. cholerae* and other *Vibrio* species from feces.[1-3,6,7]

TCBS Agar Deeps (pour tubes) are provided in a 20 mL fill so that the medium may be liquefied and poured into a Petri dish. This provides a convenient source of medium with a longer shelf-life than pre-poured plated media.

Principles of the Procedure

TCBS Agar is highly selective for the isolation of *V. cholerae* and *V. parahaemolyticus* as well as other vibrios. Inhibition of gram-positive bacteria is achieved by the incorporation of oxgall, which is a naturally occurring substance containing a mixture of bile salts, and sodium cholate, a pure bile salt. Sodium thiosulfate serves as a sulfur source and, in combination with ferric citrate, detects hydrogen sulfide production. Saccharose (sucrose) is included as a fermentable carbohydrate for the metabolism of vibrios. The alkaline pH of the medium enhances the recovery of *V. cholerae*. Thymol blue and bromthymol blue are included as indicators of pH changes.

Formula

Difco™ TCBS Agar

Approximate Formula* Per Liter

Yeast Extract	5.0	g
Proteose Peptone No. 3	10.0	g
Sodium Citrate	10.0	g
Sodium Thiosulfate	10.0	g
Oxgall	8.0	g
Saccharose	20.0	g
Sodium Chloride	10.0	g
Ferric Ammonium Citrate	1.0	g
Bromthymol Blue	0.04	g
Thymol Blue	0.04	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 89 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Cool to 45-50°C and use immediately. DO NOT AUTOCLAVE.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To prepare plated media, place agar deeps with caps loosened in a boiling water bath until the medium becomes liquefied. Pour the molten medium into a sterile Petri dish. Allow the medium to solidify. Store the plates, protected from light, in an inverted position (agar side up) at 2-8°C until ready to use.

User Quality Control

Identity Specifications
Difco™ TCBS Agar

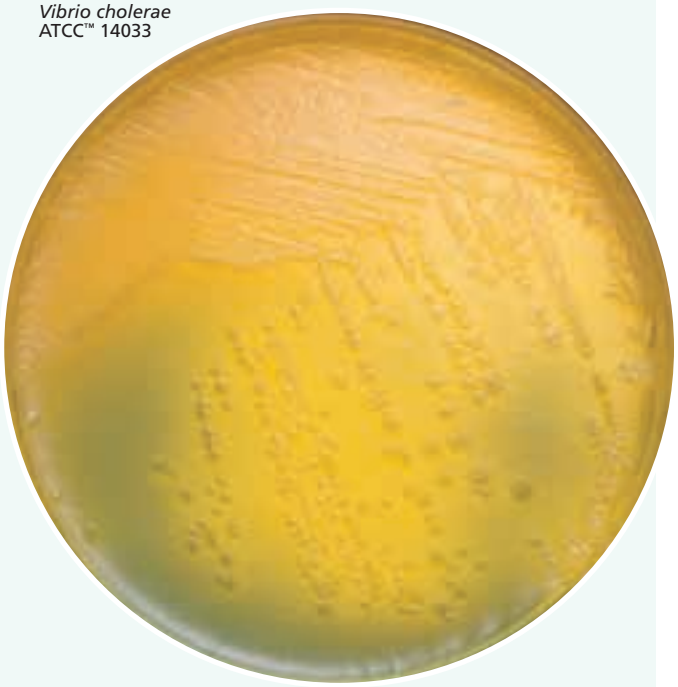
Dehydrated Appearance:	Light tan with greenish cast, free-flowing, homogeneous.
Solution:	8.9% solution, soluble in purified water upon boiling. Solution is forest green, very slightly opalescent.
Prepared Appearance:	Green, slightly opalescent.
Reaction of 8.9% Solution at 25°C:	pH 8.6 ± 0.2

Cultural Response
Difco™ TCBS Agar

Prepare the medium per label directions. Inoculate with fresh cultures (*E. coli* grown in TSB; vibrios grown in BHI) and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	RECOVERY	COLONY COLOR
Escherichia coli	25922	None	–
Vibrio alginolyticus	17749	Good	Yellow
Vibrio cholerae El Tor	14033	Good	Yellow
Vibrio parahemolyticus	17802	Good	Blue green

Vibrio cholerae
ATCC™ 14033



Use standard procedures to obtain isolated colonies from specimens. Incubate the plates, protected from light, in an inverted position (agar side up) at 35°C for 24-48 hours.

Expected Results

Typical colonial morphology on TCBS Agar is as follows:

V. cholerae ..... Large yellow colonies.
V. parahaemolyticus ..... Colonies with blue to green centers.
V. alginolyticus ..... Large yellow colonies.
Proteus/Enterococci ..... Partial inhibition. If growth, colonies are small and yellow to translucent.
Pseudomonas/Aeromonas ..... Partial inhibition. If growth, colonies are blue.

Limitations of the Procedure

1. On initial isolation, *V. parahaemolyticus* may be confused with *Aeromonas hydrophila*, *Plesiomonas shigelloides* and *Pseudomonas* species.[8]
2. Sucrose-fermenting *Proteus* species produce yellow colonies which may resemble those of *Vibrio*.[9]
3. TCBS is an unsatisfactory medium for oxidase testing of *Vibrio* spp.[10]
4. A few strains of *V. cholerae* may appear green or colorless on TCBS due to delayed sucrose fermentation.[9]

References

1. Downes and Ito. 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed, online. American Public Health Association, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Kobayashi, Enomoto, Sakazaki and Kuwahara. 1963. Jap. J. Bacteriol. *18*: 387.
5. Nakanishi. 1963. Modern Media 9: 246.
6. Furniss, Lee and Donovan. 1978. The vibrios. Public Health Laboratory Service Monograph Series no. 11. Maidstone Public Health Laboratory. H.M.S.O., London, England.
7. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc. St. Louis, Mo.
8. Bottone and Robin. 1978. J. Clin. Microbiol. 8:760.
9. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
10. Morris, Merson, Huq, Kibrya and Black. 1979. J. Clin. Microbiol. 9:79.

Availability

Difco™ TCBS Agar

AOAC | BAM | BS12 | CCAM | CMPH2 | COMPF | ISO | MCM9 | SMWW

Cat. No. 265020 Dehydrated – 500 g

BBL™ TCBS Agar

AOAC | BAM | BS12 | CCAM | CMPH2 | COMPF | ISO | MCM9 | SMWW

United States and Canada
Cat. No. 221872 Prepared Plates – Pkg. of 10*
297437 Prepared Pour Tubes, 20 mL – Pkg. of 10*

Europe
Cat. No. 254432 Prepared Plates – Pkg. of 20*

Japan
Cat. No. 251143 Prepared Plates – Pkg. of 20*
251137 Prepared Plates – Ctn. of 100*
251509 Prepared **RODAC**™ Plates – Pkg. of 30*

Mexico
Cat. No. 226850 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

m TEC Agar

Intended Use

m TEC Agar is used for isolating, differentiating and rapidly enumerating thermotolerant *Escherichia coli* from water by membrane filtration and an *in situ* urease test.

Summary and Explanation

m TEC is an acronym for "membrane Thermotolerant *E. coli*." *Escherichia coli* is widely used as an indicator of fecal pollution in water, and there are many procedures for enumerating *E. coli* based on its ability to grow at elevated temperatures and produce indole from tryptophan.[1,2] The determination of indole production in conjunction with the most-probable-number procedure often requires the use of another medium and additional incubation time.

In 1981, Dufour et al. developed a simple, accurate, nonlethal membrane filter technique for the rapid enumeration of *E. coli*.[3] This medium, m TEC Agar, quantifies *E. coli* within 24 hours without requiring subculture and identification of isolates. The authors reported that they were able to recover *E. coli* from marine, estuarine and fresh water samples.

m TEC Agar and urea substrate are recommended for use in the detection of *E. coli* when evaluating the microbiological quality of recreational waters.[4,5]

Principles of the Procedure

m TEC Agar contains sufficient nutrients to support the growth of *E. coli*. Peptone is a source of nitrogen, amino acids, carbon and amino acids. Yeast extract provides trace elements, vitamins and amino acids. Monopotassium phosphate and dipotassium phosphate offer buffering capabilities. Lactose is a fermentable carbohydrate and carbon source. Sodium lauryl sulfate and sodium desoxycholate are selective against gram-positive bacteria. Bromcresol purple and bromphenol red are indicator components. Agar is the solidifying agent.

Formula

Difco™ m TEC Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	5.0	g
Yeast Extract	3.0	g
Lactose	10.0	g
Sodium Chloride	7.5	g
Monopotassium Phosphate	1.0	g
Dipotassium Phosphate	3.3	g
Sodium Lauryl Sulfate	0.2	g
Sodium Desoxycholate	0.1	g
Bromcresol Purple	0.08	g
Bromphenol Red	0.08	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45.3 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. (Cool to 45-50°C and dispense 4-5 mL amounts into 50 × 10 mm Petri dishes and allow to solidify; store in the refrigerator.)
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ m TEC Agar

Dehydrated Appearance:	Green to grayish tan, free-flowing, homogeneous.
Solution:	4.53% solution, soluble in purified water upon boiling. Solution is deep purple with red cast, slightly opalescent.
Prepared Appearance:	Deep purple with red cast, slightly opalescent.
Reaction of 4.53% Solution at 25°C:	pH 7.3 ± 0.2

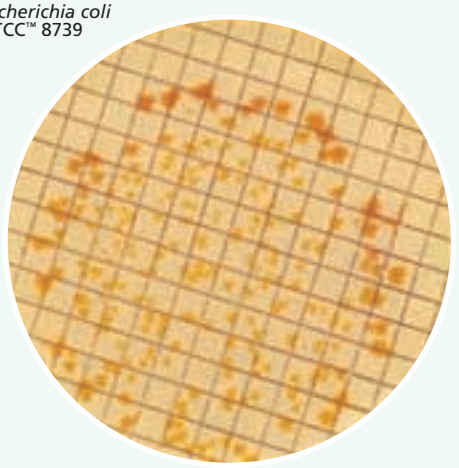
Cultural Response

Difco™ m TEC Agar

Prepare the medium per label directions. Inoculate using the membrane filter technique and incubate the plates at 35 ± 2°C for 2 hours. Transfer plates and incubate at 44.5 ± 0.5°C for 22 ± 2 hours. After incubation, remove filters and place over pads saturated with approximately 2 mL of urease substrate. Count yellow to yellow-brown colonies (urease negative) after 15-20 minutes.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	8739	20-80	Good	Yellow to yellow-brown

Escherichia coli
ATCC™ 8739



Procedure

1. Follow applicable membrane filter procedures.[4,5]
2. Incubate inoculated plates for 2 hours at 35 ± 2°C to resuscitate injured cells.
3. Transfer the plates to a 44.5 ± 0.5°C waterbath or incubator and incubate for 22 ± 2 hours.
4. Transfer countable filters to pads saturated with urea substrate. Prepare urea substrate by combining 2 g urea and 10 mg phenol red in 100 mL of purified water and adjust the pH to 5.0 ± 0.2. Store at 2-8°C and use within 1 week.

NOTE: Other methods may recommend an alternative pH.[4,5] Prepare substrate according to recommended guidelines.

5. After 15-20 minutes, count all yellow to yellow-brown colonies with the aid of a stereoscopic microscope.

Expected Results

Yellow to yellow-brown colonies (urease negative) may be presumptively identified as *E. coli*.

Limitations of the Procedure

1. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
2. The urease test is required to presumptively identify *E. coli*.
3. Choose a water sample size that will result in 20-80 colonies per filter. Plates containing more than 80 colonies are not recommended because high counts may not provide accurate urease test results.
4. Do not trap air bubbles underneath the filter.

References

1. Mara. 1973. J. Hyg. *71*:783.
2. Pugsley, Evision and James. 1973. Water Res. *7*:1431.
3. Dufour, Strickland and Cabelli. 1981. Appl. Environ. Microbiol. *41*:1152.
4. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater. 21st ed., online. American Public Health Association, Washington, D.C.
5. American Society for Testing and Materials. 1996. Annual Book of ASTM Standards. Water and Environmental Technology (PCN: 01-110296-16). ASTM, West Conshohocken, Pa.

Availability

Difco™ m TEC Agar

SMWW

Cat. No. 233410 Dehydrated – 100 g

mTEC Agar, Modified

Intended Use

Modified mTEC Agar is a selective culture medium used for the chromogenic detection and enumeration of thermotolerant *Escherichia coli* in water by the membrane filtration technique. It conforms with U.S. Environmental Protection Agency (USEPA) Approved Method 1603: Escherichia coli (E. coli) *in Water by Membrane Filtration Using Modified membrane-Thermotolerant* Escherichia coli *Agar (modified mTEC)*.

Summary and Explanation

mTEC is an acronym for "membrane Thermotolerant *E. coli*." *E. coli* is widely used as an indicator of fecal pollution in water. This organism has a high correlation with gastroenteritis in fresh water environments.[1] In 1986, the USEPA recommended that *E. coli* be used as a bacterial water quality indicator to monitor recreational waters.[2]

Many procedures have been developed for enumerating *E. coli* based on its ability to grow at elevated temperatures and produce indole from tryptophan.[3,4] The determination of indole production in conjunction with the most-probable-number procedure often requires the use of another medium and additional incubation time.

Dufour developed a membrane filtration procedure using mTEC agar for the rapid enumeration of thermotolerant *E. coli*.[5,6] This alternative two-step test procedure quantified *E. coli* within 24 hours without requiring subculture and identification of isolates. However, the membrane filter had to be transferred after initial incubation at an elevated temperature to a urea substrate/phenol red-saturated pad.

Modified mTEC Agar was developed by the USEPA in 1998[7,8] as a single-step procedure that does not require the transfer of the membrane filter to another substrate. The modified medium contains the chromogen, 5-bromo-6-chloro-3-indolyl-β-D-glucuronide. This chromogen is catabolized to glucuronic acid by *E. coli* strains that produce the enzyme β-D-glucuronidase to form red- or magenta-colored colonies, enabling confirmatory identification of *E. coli* in 24 hours. Red or magenta colonies can be verified as *E. coli* in instances where required in evidence gathering or for performing quality control for the initial use of this test.[8]

This medium is recommended for testing the presence of *E. coli* as an indicator organism for fecal contamination in fresh recreational water. This allows for a wide range of sample volumes or dilutions to be analyzed by membrane filtration for the detection and enumeration of *E. coli* levels in water. The USEPA-published false-positive rate is <1% and false-negative rate is 4% from a variety of environmental water samples.[8]

Principles of the Procedure

Modified mTEC Agar contains sufficient nutrients to support the growth of *E. coli*. Peptone is a source of nitrogen, amino acids, carbon and amino acids. Yeast extract provides trace elements, vitamins and amino acids. Lactose is a fermentable carbohydrate and carbon source. Sodium chloride maintains osmotic equilibrium. Monopotassium and dipotassium phosphates offer buffering capabilities. Sodium lauryl sulfate and sodium desoxycholate are selective against gram-positive bacteria. The chromogen, 5-bromo-6-chloro-3-indolyl-β-D-

User Quality Control

Identity Specifications

Difco™ Modified mTEC Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.56% solution, soluble in purified water upon boiling. Solution is light to medium tan, very slightly to slightly opalescent, without significant precipitate.
Prepared Appearance:	Light tan, clear to very slightly opalescent, without significant precipitate. Upon removal from 2-8°C storage, plates may exhibit a crystal precipitate that disappears upon warming to room temperature. This is a typical characteristic of the medium and is acceptable.
Reaction of 4.56% Solution at 25°C:	pH 7.3 ± 0.2

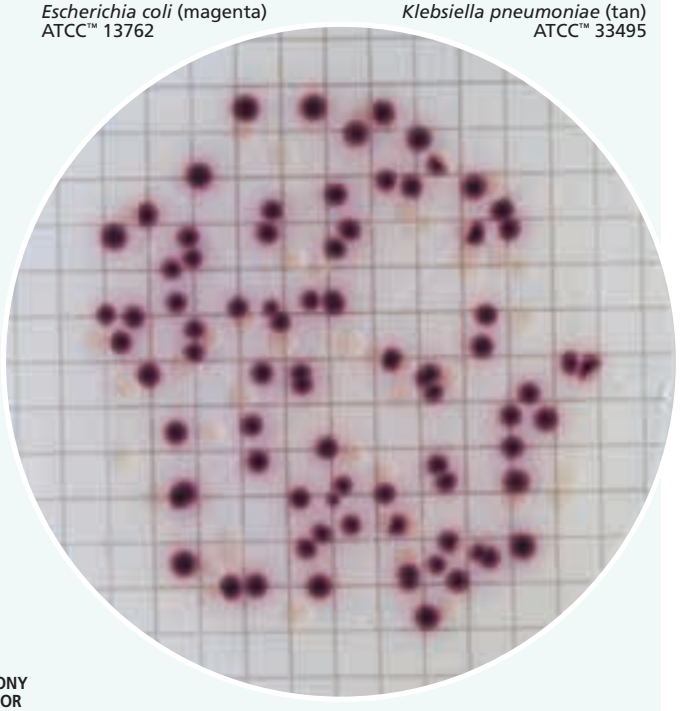
Cultural Response

Difco™ Modified mTEC Agar

Prepare the medium per label directions. Inoculate using the membrane filtration technique and incubate at 35°C for 2 hours. Transfer plates and incubate at 44.5 ± 0.2°C for approximately 22-24 hours. Count all red or magenta colonies.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	19433	20-80	Marked to complete inhibition	–
Escherichia coli	13762	20-80	Good	Red or magenta
Proteus mirabilis	25933	20-80	Good	Tan

Escherichia coli (magenta) ATCC™ 13762 — *Klebsiella pneumoniae* (tan) ATCC™ 33495



glucuronide, is catabolized to form glucuronic acid and a red- or magenta-colored compound by *E. coli* that produce the enzyme β-D-glucuronidase. Agar is the solidifying agent.

Formula

Difco™ Modified mTEC Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	5.0	g
Yeast Extract	3.0	g
Lactose	10.0	g
Sodium Chloride	7.5	g
Dipotassium Phosphate	3.3	g
Monopotassium Phosphate	1.0	g
Sodium Lauryl Sulfate	0.2	g
Sodium Desoxycholate	0.1	g
5-Bromo-6-chloro-3-indolyl-β-D-glucuronide	0.5	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45.6 g of the powder in 1 L purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C in a water bath.
4. Determine pH of medium (remove an aliquot and cool to room temperature) and adjust pH to 7.3 ± 0.2 by aseptically adding sterile 1N NaOH.
5. Dispense 5 mL amounts into 9 × 50 mm or 15 × 60 mm plates and allow to solidify.
6. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Upon removal from 2-8°C storage, plates may exhibit a crystal precipitate that disappears upon warming to room temperature. This is a typical characteristic of the medium and is acceptable.

Procedure

1. Collect and prepare water samples in accordance with recommended guidelines.[9,10]
2. Test required sample volumes following the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.[9] Select sample volumes to produce 20-80 colonies on the membrane filter.
3. After sample has been filtered, aseptically remove membrane filter from filter base and roll it onto Modified mTEC Agar to avoid the formation of bubbles between the membrane and the agar surface.
4. Invert inoculated plates and incubate for 2 hours at 35 ± 0.5°C to resuscitate injured cells.
5. After a 2-hour incubation at 35 ± 0.5°C, transfer the plates to a plastic bag, seal the bag, and place it onto a rack in a 44.5 ± 0.2°C water bath for 22 - 24 hours.
6. After the 22-24 hour incubation, remove the plates from the water bath and count and record the number of red or

magenta colonies using an illuminated lens with a 2-5× magnification or a stereoscopic microscope.
7. Calculate and report the number of *E. coli* colonies per 100 mL of sample.

Expected Results

Red to magenta colonies may be presumptively identified as *E. coli*. Refer to the USEPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.[11]

Limitations of the Procedure

1. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
2. Choose a water sample size that will result in 20-80 colonies per filter. Plates containing more than 80 colonies are not recommended because high counts may not provide accurate test results.
3. Minimize the exposure of Modified mTEC Agar to light before and during incubation, as light may destroy the chromogen.

References

1. U.S. Environmental Protection Agency. 1986. Ambient water quality criteria for bacteria - 1986. Publication EPA-440/5-84/002. Office of Water, Regulations and Standards. Criteria and Standards Division, USEPA, Washington, D.C.
2. U.S. Environmental Protection Agency. 1986. Bacteriological ambient water quality criteria: availability. Fed. Reg. *51*(45):8012.
3. Mara. 1973. J. Hyg. *71*:783.
4. Pugsley, Evision, and James. 1973. Water Res. 7:1431.
5. Eaton, Clesceri, and Greenberg (ed.). 1995. Standard methods for the examination of water and wastewater. 19th ed. American Public Health Association, Washington, D.C.
6. Dufour, Strickland and Cabelli. 1981. Appl. Environ. Microbiol. *41*:1152.
7. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicator: enterococci and *Escherichia coli*. Publication EPA/821/R-97/004. Office of Science and Technology, USEPA, Washington, D.C.
8. U.S. Environmental Protection Agency. 2002. Method 1603: *Escherichia coli (E. coli)* in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar (modified mTEC). Publication EPA-821-R-02-023. USEPA Office of Water, Office of Science and Technology, Washington, DC.
9. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
10. ASTM International. 2002. Annual book of ASTM standards. Water and environmental technology. ASTM International, West Conshohocken, Pa.
11. Bordner, Winter and Scarpino (ed.). 1978. Microbiological methods for monitoring the environment: water and wastes. Publication EPA-600/8-78/017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Availability

Difco™ Modified mTEC Agar

EPA

Cat. No.	214884	Dehydrated – 100 g
	214880	Dehydrated – 500 g

BBL™ Modified mTEC Agar

EPA

Cat. No.	215044	Prepared Plates – Pkg. of 20*
	215046	Prepared Plates – Ctn.of 100*

**Store at 2-8°C.*

T

TSN Agar

Intended Use

TSN (**Trypticase**™ Sulfite Neomycin) Agar is used for the selective isolation of *Clostridium perfringens*.

Summary and Explanation

TSN Agar was developed by Marshall et al. as a medium that could achieve rapid enumeration of *Clostridium perfringens*.[1] The formulation is a modification of Mossel's medium for the enumeration of sulfite-reducing clostridia in foods.[2] The 46°C temperature of incubation for TSN Agar permits specific and quantitative results.

User Quality Control

Identity Specifications

BBL™ TSN Agar

Dehydrated Appearance:	Fine, homogeneous, may contain a large amount of minute to small dark particles.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to moderately hazy.
Reaction of 4.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ TSN Agar

Prepare the medium per label directions. Inoculate and incubate at 46 ± 1°C anaerobically for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
Clostridium bifermentans	17836	Undiluted	Partial to complete inhibition	With or without blackening
Clostridium perfringens	3624	Undiluted	Good	Blackening
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10^4-10^5	Partial to complete inhibition	No blackening

Principles of the Procedure

Neomycin and polymyxin are inhibitory for gram-negative enteric bacilli. Neomycin at the concentration employed at least partially inhibits *C. bifermentans*. The relatively high incubation temperature of 46°C renders the medium highly specific for *C. perfringens*. The colonies are black due to the formation of ferric sulfide as a result of the reduction of the sulfite.

Formula

BBL™ TSN Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Sodium Sulfite	1.0	g
Neomycin Sulfate	0.05	g
Polymyxin Sulfate	0.02	g
Yeast Extract	10.0	g
Ferric Citrate	0.5	g
Agar	13.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 118°C for 12 minutes. Do not overheat.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use on the day of preparation. Inoculate tubes or plates of the medium by stabbing deep tubes or streaking plates with the test specimen. Incubate containers for 18-24 hours at 46 ± 0.1°C in an anaerobic atmosphere (**BD GasPak™** EZ anaerobic system or equivalent).

Expected Results

C. perfringenes produces black colonies at 46°C. *C. perfringens* and *C. bifermentans* produce black colonies on TSN Agar at 37°C; however, *C. bifermentans* is inhibited at 46°C.[1]

References

1. Marshall, Steenbergen and McClung. 1965. Appl. Microbiol. *13*:559.
2. Mossel. 1959. J. Sci. Food Agric. *10*:662.

Availability

BBL™ TSN Agar

Cat. No. 211690 Dehydrated – 500 g

TT Broth Base, Hajna

Intended Use

TT (Tetrathionate) Broth Base, Hajna is used for enriching *Salmonella* from food and dairy products prior to isolation procedures.

Summary and Explanation

TT Broth Base, Hajna is used as a selective enrichment for the cultivation of *Salmonella* spp. *Salmonella* organisms can be injured in food-processing procedures. These procedures include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives and sanitizers.[1] Although injured cells may not form colonies on selective media, they can cause disease if ingested.[2] *Salmonella* spp., in particular, cause many types of infections from mild self-limiting gastroenteritis to life-threatening typhoid fever.[3] The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting less than 7 days.[3]

TT Broth Base, Hajna conforms to the formulation of Hajna and Damon.[4] The medium is a modification of the enrichment described by Kauffmann[5] and Knox.[6] Hajna and Damon[4] developed a new broth containing yeast extract, peptone, carbon sources and the selective agents, sodium desoxycholate and brilliant green (replacing bile salts).

TT Broth Base, Hajna is used in testing *Salmonella* in egg processing plants.[7] It is included in procedures for the isolation and identification of *Salmonella* from meat and poultry as well as egg products.[8]

Principles of the Procedure

Peptone provides nitrogen and amino acids. Yeast extract supplies growth factors and vitamins. Dextrose and mannitol are fermentable carbohydrates. Selectivity is accomplished by the combination of sodium thiosulfate and tetrathionate, suppressing coliform organisms.[6] Tetrathionate is formed in the medium by the addition of a solution containing iodine and potassium iodide. Organisms containing the enzyme tetrathionate reductase will proliferate in this medium.

Sodium desoxycholate and brilliant green are selective agents that suppress coliform bacteria and inhibit gram-positive organisms. Sodium chloride maintains the osmotic balance of the medium. Calcium carbonate is a neutralizer that absorbs toxic metabolites.

Formula

Difco™ TT Broth Base, Hajna

Approximate Formula* Per Liter		
Yeast Extract	2.0	g
Tryptose	18.0	g
Dextrose	0.5	g
D-Mannitol	2.5	g
Sodium Desoxycholate	0.5	g
Sodium Chloride	5.0	g
Sodium Thiosulfate	38.0	g
Calcium Carbonate	25.0	g
Brilliant Green	0.01	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ TT Broth Base, Hajna

Dehydrated Appearance:	Beige to very light green, free-flowing, homogeneous.
Solution:	9.15% solution, partially insoluble in purified water upon boiling. Solution is light green, slightly opalescent with a heavy white precipitate.
Prepared Appearance:	Light green, slightly opalescent with a heavy white precipitate.
Reaction of 9.15% Solution at 25°C:	pH 7.6 ± 0.2 (after addition of the iodine solution)

Cultural Response

Difco™ TT Broth Base, Hajna

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, plate the inoculated broth onto MacConkey Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR ON MACCONKEY AGAR
Escherichia coli	25922	10^2-10^3	None to poor	Pink with bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Colorless

Directions for Preparation from Dehydrated Product

1. Suspend 91.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat to boiling. DO NOT AUTOCLAVE. Cool to below 50°C.
3. Add 40 mL iodine solution (5 g iodine crystals and 8 g potassium iodide dissolved in 40 mL of purified water) and mix well.
4. Dispense into sterile tubes while keeping suspension well mixed. Do not heat the medium after adding iodine.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

After preparation, add 1-3 g of fecal specimen to each tube (heavy inoculum). Incubate tubes for 12-24 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Growth is indicated by turbidity in the medium. Subculture to selective and differential enteric plating media for further investigations.

References

1. Hartman and Minnich. 1981. J. Food Prot. *44*:385.
2. Sorrells, Speck and Warren. 1970. Appl. Microbiol. *19*:39.
3. Gray. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. Hajna and Damon. 1956. Appl. Microbiol. *4*:341.
5. Kauffman. 1930. Zentralb. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abr. I Orig. *113*:148.
6. Knox, Gell and Pollack. 1942. J. Pathol. Bacteriol. *54*:469.
7. Catalano and Knable. 1994. J. Food Prot. *57*:587.
8. U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Difco™ TT (Tetrathionate) Broth Base, Hajna

USDA

Cat. No.	249120	Dehydrated – 500 g
	249110	Dehydrated – 2 kg

Tech Agar

(See Pseudomonas Agars)

Tellurite Glycine Agar Tellurite Solution 1%

Intended Use

Tellurite Glycine Agar is used with Tellurite Solution 1% for isolating coagulase-positive staphylococci.

Summary and Explanation

Coagulase-positive *Staphylococcus aureus* is well documented as a human opportunistic pathogen.[1] Foods are examined for the presence of *S. aureus* and/or its enterotoxins to confirm that *S. aureus* is the causative agent of foodborne illness, to determine whether a food is the source of "staph" food poisoning and to determine post-processing contamination.[2]

User Quality Control

Identity Specifications

Difco™ Tellurite Glycine Agar

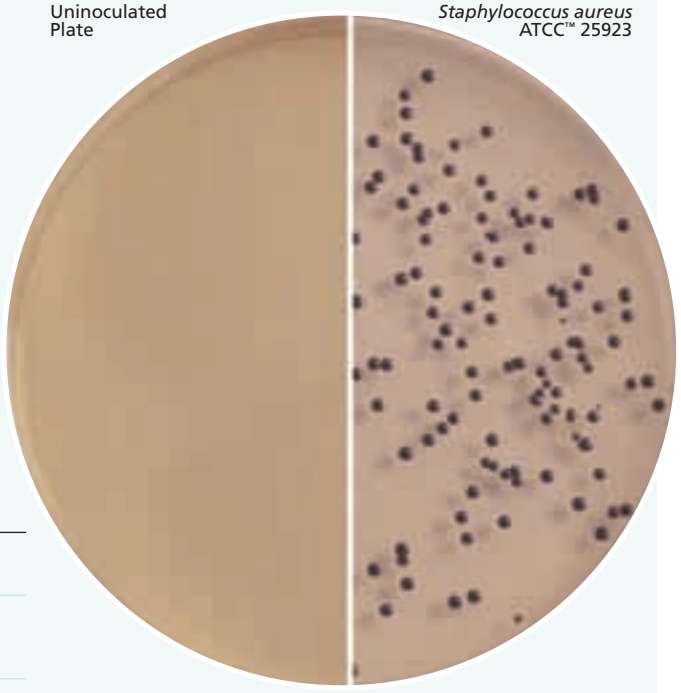
Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.25% solution, soluble in purified water upon boiling. Solution is amber, opalescent with precipitate.
Prepared Appearance:	Medium amber, opalescent with precipitate.
Reaction of 6.25% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Tellurite Glycine Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	30-300	Marked to complete inhibition	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Marked to complete inhibition	–
Staphylococcus aureus	25923	30-300	Good	Black
Staphylococcus epidermidis	12228	30-300	Partial inhibition	Gray, if any



Ludlam[3] described a selective medium for the isolation of staphylococci. This medium was alkaline in reaction, contained mannitol, and lithium chloride with potassium tellurite as the selective agents. Zebovitz, Evans and Niven[4] modified Ludlam's medium by adding glycine as a selective agent and adjusting the reaction of the basal medium to pH 7.2 instead of pH 9.6.

Tellurite Glycine Agar is prepared according to the formula of Zebovitz, Evans and Niven.[4] The medium permits the isolation of coagulase-positive staphylococci from food, air, dust, soil and clinical specimens. Coagulase-negative staphylococci and other bacteria are markedly to completely inhibited.

Principles of the Procedure

Peptones are sources of nitrogen and amino acids in Tellurite Glycine Agar. Yeast extract is a vitamin source in this formulation. D-Mannitol is a source of fermentable carbohydrate for coagulase-positive staphylococci. Lithium chloride, glycine and potassium tellurite are the selective agents. Dipotassium phosphate is used to buffer the medium. Agar is the solidifying agent.

Tellurite Solution is a sterile 1% solution of potassium tellurite, a differential agent. Coagulase-positive staphylococci reduce tellurite and produce black colonies.[5]

Formula

Difco™ Tellurite Glycine Agar

Approximate Formula* Per Liter		
Yeast Extract	6.5	g
Soytone	3.5	g
Tryptone	10.0	g
Glycine	10.0	g
D-Mannitol	5.0	g
Dipotassium Phosphate	5.0	g
Lithium Chloride	5.0	g
Agar	17.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 62.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add 10 mL Tellurite Solution 1% to the medium at 50-55°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the isolation and identification of coagulase-positive staphylococci from clinical specimens refer to appropriate procedures.[1,6] For the examination of staphylococci in foods refer to standard methods.[2,7]

Expected Results

Coagulase-positive staphylococci produce black colonies within 24 hours of incubation at 35°C.

Limitation of the Procedure

Occasional coagulase-negative staphylococci may produce small gray colonies, not readily confused with black coagulase-positive colonies.

References

1. Bannerman and Peacock. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.). Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
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5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, Md.
6. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
7. Lancette and Bennett. 2001. *In* Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Tellurite Glycine Agar

Cat. No. 261710 Dehydrated – 500 g

BBL™ Tellurite Solution 1%

Cat. No. 211917 Tube – 1 × 20 mL*

**Store at 2-8°C.*

Terrific Broth

Intended Use

Terrific Broth is used with glycerol in cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

Terrific Broth is a highly enriched medium developed by Tartoff and Hobbs to improve yield in plasmid-bearing *E. coli*.[1] Recombinant strains have an extended growth phase in the medium. The addition of extra peptone and yeast extract in the medium allows higher plasmid yield per volume. Glycerol is used as the carbohydrate source in this formulation. Unlike glucose, glycerol is not fermented to acetic acid.

Principles of the Procedure

Peptone and yeast extract provide necessary nutrients and cofactors for excellent growth of recombinant strains of *E. coli*. The yeast extract concentration is increased to allow for elevated cell yields. Potassium phosphates are added to provide potassium for cellular systems and prevent cell death due to a drop in pH. Glycerol is added as a carbon and energy source.

User Quality Control

Identity Specifications

Difco™ Terrific Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.76% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 4.76% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Terrific Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli (C600)	23724	10^2-10^3	Good
Escherichia coli (HB101)	33694	10^2-10^3	Good
Escherichia coli (DH-1)	33849	10^2-10^3	Good
Escherichia coli (JM103)	39403	10^2-10^3	Good
Escherichia coli (JM107)	47014	10^2-10^3	Good
Escherichia coli (DH-5)	53868	10^2-10^3	Good

Formula

Difco™ Terrific Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	12.0	g
Yeast Extract	24.0	g
Dipotassium Phosphate	9.4	g
Monopotassium Phosphate	2.2	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 47.6 g of the powder in 1 L of purified water.
2. Add 4 mL of glycerol to the medium.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[1,2]

Expected Results

Growth is evident in the form of turbidity.

References

1. Tartoff and Hobbs. 1987. Bethesda Research Laboratories Focus 9:12.
2. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Availability

Difco™ Terrific Broth

Cat. No. 243820 Dehydrated – 500 g
243810 Dehydrated – 2 kg

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

Tetrathionate Broth Base

Intended Use

Tetrathionate Broth Base, with added iodine-iodide solution, is used as a selective enrichment medium for the isolation of *Salmonella* from feces, urine, foods and other materials of sanitary importance.

Summary and Explanation

Tetrathionate Broth Base is used as a selective enrichment for the cultivation of *Salmonella* species that may be present in small numbers and compete with intestinal flora. *Salmonella* organisms may also be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives and sanitizers.[1] Although injured cells may not form colonies on selective media, they can, if ingested, cause disease.[2]

Tetrathionate Broth was originally described by Mueller who found that the medium selectively inhibited coliforms, thereby permitting enteric pathogens to grow virtually without restriction.[3] Kaufmann modified Mueller's medium and achieved a higher percentage of isolates.[4,5] The medium is specified in standard methods.[6,7]

Principles of the Procedure

Peptones provide nitrogen, vitamins, amino acides and carbon. Oxgall inhibits gram-positive microorganisms. Tetrathionate, which is formed in the medium by the addition of the iodine-iodide solution, inhibits the normal intestinal flora of fecal specimens.[8] Calcium carbonate neutralizes and absorbs toxic metabolites.

Formula

Difco™ Tetrathionate Broth Base

Approximate Formula* Per Liter		
Proteose Peptone	2.5	g
Pancreatic Digest of Casein	2.5	g
Oxgall	1.0	g
Sodium Thiosulfate	30.0	g
Calcium Carbonate	10.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 4.6 g of the powder in 100 mL of purified water.
2. Heat to boiling. Cool to below 60°C.
3. Add 2 mL of iodine solution (6.0 g of iodine crystals and 5.0 g of potassium iodide in 20.0 mL of water). DO NOT REHEAT MEDIUM AFTER ADDING IODINE. DO NOT AUTOCLAVE.
4. Use immediately.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For tubes prepared from dehydrated media with added iodine, inoculate with a swab or loopful of specimen or, where the tube volume permits, add feces, other solid sample or liquid specimen (approximately 10% by volume) and emulsify with an inoculating needle, if necessary. Incubate tubes for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

User Quality Control

Identity Specifications

Difco™ Tetrathionate Broth Base

Dehydrated Appearance:	White to off-white, may have a slight greenish tint, free-flowing, homogeneous.
Solution:	4.6% solution, partially insoluble in purified water. Solution is a milky white, opaque suspension. Upon standing, appearance is nearly colorless to light yellow supernatant over insoluble white precipitate.
Prepared Appearance:	Nearly colorless to light yellow supernatant over a heavy white precipitate.
Reaction of 4.6% Solution at 25°C:	pH 8.4 ± 0.2

Cultural Response

Difco™ Tetrathionate Broth Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, subculture onto MacConkey Agar plates and incubate plated media at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONIES ON MACCONKEY AGAR
Escherichia coli	25922	10^2-10^3	Little or no increase in number of colonies	Pink with bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Colorless

For **BBL**™ Tetrathionate Broth Base prepared tubes, prepare an iodine-iodide solution by adding 6.0 g of iodine crystals and 5.0 g of potassium iodide to 20.0 mL of sterile purified water. Immediately before inoculation, add 0.2 mL of the iodine-iodide solution to each 10 mL of medium and inoculate as described above.

Expected Results

Growth is indicated by turbidity in the medium. Subculture to selective and differential enteric plating media for further investigations.

Limitation of the Procedure

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult references for detailed information and recommended procedures.[6,7,9]

References

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3. Mueller. 1923. C. R. Soc. Biol. (Paris) *89*:434.
4. Kaufman. 1930. Zentrabl. Bakteriol. Parsitenkd. Infektionskr. Hyg. Abt. I Orig. *113*:148.
5. Kaufman. 1935. Z. Hyg. Infektionskr. *117*:26.
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7. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
8. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, Md.
9. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Tetrathionate Broth Base

AOAC | BAM | CCAM | COMPF | SMD | SMWW

Cat. No.	210430	Dehydrated – 500 g
	210420	Dehydrated – 2 kg

BBL™ Tetrathionate Broth Base

AOAC | BAM | CCAM | COMPF | SMD | SMWW

Cat. No.	298249	Prepared Tubes, 10 mL (D Tubes) – Pkg. of 10*

Europe

Cat. No.	257329	Prepared Bottles, 5 mL – Pkg. of 50*
	257328	Prepared Bottles, 10 mL – Pkg. of 50*
	254958	Prepared Bottles, 100 mL – Pkg of 25*

**Store at 2-8°C.*

Thayer-Martin Selective Agar Modified Thayer-Martin (MTM II) Agar

Intended Use

Thayer-Martin Selective Agar and Modified Thayer-Martin (MTM II) Agar are used for the isolation of pathogenic *Neisseria* from specimens containing mixed flora of bacteria and fungi.

Summary and Explanation

Thayer-Martin Selective Agar was developed for the primary isolation of *N. gonorrhoeae* and *N. meningitidis* from specimens containing mixed flora taken from the throat, vagina, rectum and urethra.[1-3] Consisting of Chocolate II Agar with vancomycin, colistin and nystatin, it is formulated to minimize the overgrowth of gonococci and meningococci by contaminants, to suppress the growth of saprophytic *Neisseria* species and to enhance the growth of pathogenic *Neisseria*.

Martin et al. modified Thayer-Martin Selective Agar by adding trimethoprim to produce Modified Thayer-Martin Agar.[4] A significantly greater number of positive gonococcal isolates from clinical specimens were reported as compared with Thayer-Martin Selective Agar due to the inhibition of swarming *Proteus* species.[4-6] Because of its improved performance, it is recommended over earlier formulations for the isolation of *N. gonorrhoeae*.[7,8] The original formula contained 20 g/L of agar and 1.5 g/L dextrose (in addition to the dextrose in the **BBL**™ **IsoVitaleX**™ Enrichment). The agar concentration has been changed to approximately 12 g/L; the extra 1.5 g/L of dextrose has been eliminated since the lower dextrose content was found to improve the growth of *N. gonorrhoeae*. **BBL** MTM II was developed by careful selection and pretesting of raw materials to provide enhanced growth of gonococci as well as improved inhibition of *Candida* species.

Gono-Pak is the name given to a selective medium-resealable polyethylene bag-CO_2 generating tablet system described by Holston et al. for the isolation of *N. gonorrhoeae*. It was found to be comparable to the candle jar method for the isolation of *N. gonorrhoeae* from clinical specimens.[9,10] The Gono-Pak system obviates the need both for a separate carbon dioxide system and for transferring the specimen from the transport system to the culture plate. It has been reported to be superior to Transgrow (Modified Thayer-Martin Agar with CO_2-enriched atmosphere in a bottle) as a transport system.[11]

The JEMBEC™* style plate was developed by John E. Martin, Jr., of the Centers for Disease Control in association with Ames Laboratories and was designed to provide a self-contained CO_2 environment through the use of a CO_2-generating tablet placed in a specially designed well provided in the plate.[12]

The JEMBEC system is recommended for the growth and transportation of *Neisseria gonorrhoeae* and has the advantage over other transport systems of obviating the necessity of transferring the specimen from the transport system to a culture plate.

**JEMBEC is a trademark of Miles Scientific.*

Principles of the Procedure

Thayer-Martin Selective Agar and Modified Thayer-Martin (MTM II) Agar are based on Chocolate II Agar, which contains an improved GC Agar base, bovine hemoglobin and **IsoVitaleX** Enrichment. The GC II base contains nitrogenous nutrients in the form of casein and meat peptones, phosphate buffer to maintain pH and corn starch, which neutralizes toxic fatty acids that may be present in the agar. Hemoglobin provides X factor (hemin) for *Haemophilus* spp. **IsoVitaleX** Enrichment is a defined supplement which provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

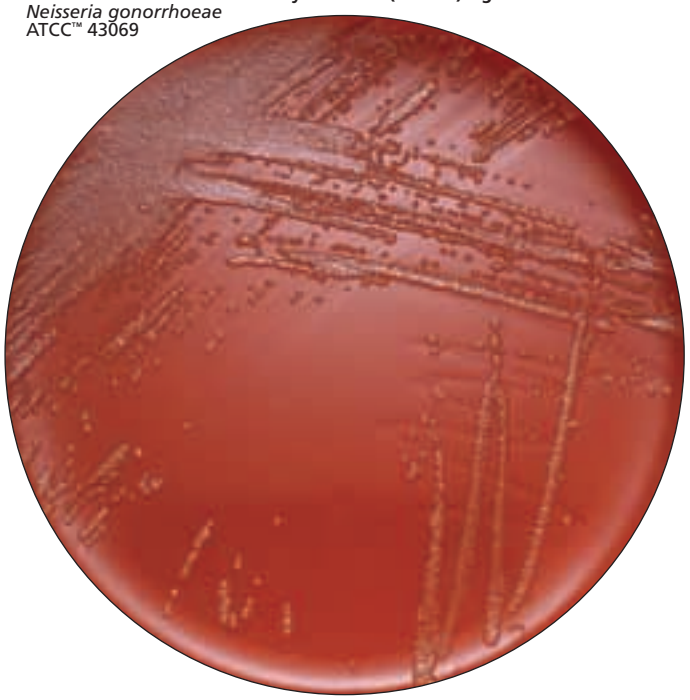
These selective media contain the antimicrobial agents, vancomycin, colistin and nystatin (V-C-N inhibitor) to suppress the normal flora. Vancomycin is active primarily against gram-positive bacteria. Colistin inhibits gram-negative bacteria, including *Pseudomonas* spp., but is not active against *Proteus* spp. Nystatin inhibits fungi.

Modified Thayer-Martin (MTM II) Agar also contains trimethoprim for the inhibition of *Proteus* spp.

In the Gono-Pak system, a tablet consisting of a mixture of citric acid and sodium bicarbonate is activated by the moisture (humidity) produced by the culture medium within the sealed plastic bag and generates CO_2 levels sufficient for the growth of *Neisseria gonorrhoeae* on the selective medium provided with the system.[10]

In the JEMBEC system, a tablet consisting of a mixture of citric acid and sodium bicarbonate is placed in a well within the plate and is activated by the moisture (humidity) produced by the culture medium within the sealed plastic bag. The CO_2 levels generated are sufficient for the growth of *Neisseria gonorrhoeae* on the selective medium provided with the system.[12]

Modified Thayer-Martin (MTM II) Agar
Neisseria gonorrhoeae
ATCC™ 43069



Procedure

Streak the specimen as soon as possible after it is received in the laboratory. If material is being cultured directly from a swab, proceed as follows:[12]

1. Roll swab directly on the medium in a large "Z" to provide adequate exposure of swab to the medium for transfer of organisms.
2. Cross-streak the "Z" pattern with a sterile wire loop, preferably in the clinic. If not done previously, cross-streaking should be done in the laboratory.
3. Place the culture as soon as possible in an aerobic environment enriched with carbon dioxide.
   a. With the Gono-Pak System:
   Place inoculated plates in the polyethylene bag provided (one or two plates per bag). Cut off the corner of one foil-wrapped CO_2 tablet to expose the tablet and place in the bag. DO NOT ADD WATER TO THE TABLET.
   To seal the bag, simply press down on the "zipper" at the end of the bag with fingers and slide along to the opposite end. Be sure that the bag is sealed completely. After the bag is sealed, incubate in an inverted position (agar bed up) at 35°C for 18-48 hours.[7, 13]
   To transport the culture after incubation, place the sealed Gono-Pak system in a suitable mailing or shipping container. Care should be taken to protect the culture from extreme heat or cold and to ensure delivery to the testing laboratory as rapidly as possible.
   b. With the JEMBEC System:
   With sterile forceps, remove a CO_2-generating tablet from its foil wrapper and place it in the specially designed well in the plate. Place inoculated plates in the polyethylene bag provided (one plate per bag). DO NOT ADD WATER TO THE TABLET. Seal the bag by pressing down on the "zipper" at the end of the bag with fingers and slide along to the opposite end. Be sure that the bag is sealed completely. After the bag is sealed, incubate in an inverted position (agar bed up) at 35°C for 18-48 hours.[7,13]
   To transport the culture after incubation, place the sealed JEMBEC system in a suitable mailing or shipping container. Care should be taken to protect the culture from extreme heat or cold and to ensure delivery to the testing laboratory as soon as possible.
4. Incubate at 35 ± 2°C and examine after overnight incubation and again after approximately 48 hours.
5. Subculture for identification of *N. gonorrhoeae* should be made within 18-24 hours.

Expected Results

Typical colonial morphology on these media is as follows:

Neisseria gonorrhoeae..... Small, grayish-white to colorless, mucoid
Neisseria meningitidis..... Medium to large, blue-gray, mucoid

Colonies may be selected for Gram staining, subculturing or other diagnostic procedures.

Limitations of the Procedure

Selective media for pathogenic *Neisseria* may inhibit other pathogenic bacteria, e.g., *Haemophilus*.

The existence of strains of *N. gonorrhoeae* inhibited by the components of V-C-N Inhibitor and trimethoprim lactate have been reported.[14,15]

While "saprophytic" *Neisseria* are generally suppressed by selective media, the occasional recovery of *N. lactamica* on Thayer-Martin Selective Agar has been reported.[16]

Some strains of *Capnocytophaga* species may grow on these selective media when inoculated with oropharyngeal specimens.[17]

References

1. Martin, Billings, Hackney and Thayer. 1967. Public Health Rep. *82*:361.
2. Thayer and Martin. 1966. Pub. Health Rep. *81*:559.
3. Mitchell, Rhoden and Marcus. 1966. Am. J. Epidem. *83*:74.
4. Martin, Armstrong and Smith. 1974. Appl. Microbiol. *27*:802.
5. Center for Disease Control. January 2, 1975. Memorandum: recommendation to use the same medium, Modified Thayer-Martin (MTM), in both plates and bottles for the GC culture screening program. U. S. Public Health Service, Atlanta, Ga.
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7. Evangelista and Beilstein. 1993. Cumitech 4A, Laboratory diagnosis of gonorrhoea. Coord. ed., Abramson. American Society for Microbiology, Washington, D.C.
8. Kanpp and Koumans. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
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10. DeVaux, Evans, Arndt and Janda. 1987. J. Clin. Microbiol. *25*:571.
11. Lewis and Weisner. 1980. Lab. Management. *18*:33.
12. Center for Disease Control. 1975. Criteria and techniques for the diagnosis of gonorrhea. U. S. Public Health Service, Atlanta, Ga.
13. Lewis. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
14. Cross, Hoger, Neibaur, Pasternack and Brady. 1971. HSMHA Health Rep. *86*:990.
15. Phillips, Humphrey, Middleton and Nicol. 1972. Br. J. Vener. Dis. *48*:287.
16. Edberg. 1974. J. Clin. Pathol. *62*:445.
17. Reichart, Rupkey, Brady and Hook. 1989. J. Clin. Microbiol. *27*:808.

Availability

BBL™ Modified Thayer-Martin (MTM II) Agar

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	221567	Prepared Plates – Pkg of 20*
	221568	Prepared Plates – Ctn. of 100*

Japan

Cat. No.	252140	Prepared **I-Plate**™ Dishes – Ctn. of 100*

BBL™ Thayer-Martin Selective Agar

United States and Canada

Cat. No.	221184	Prepared Plates – Pkg of 20*
	221282	Prepared Plates – Ctn. of 100*

Japan

Cat. No.	251184	Prepared Plates – Pkg of 20*
	252084	Prepared Plates – Ctn. of 100*
	251809	Prepared Plates – Ctn. of 200*

BBL™ Modified Thayer-Martin (MTM II) Agar// Chocolate II Agar

Cat. No.	221623	Prepared Bi-Plate Dishes (100 x 15 mm-style, Divided) – Pkg. of 20*

BBL™ Modified Thayer-Martin (MTM II) Agar Gono-Pak

BS12 CMPH2 MCM9

Cat. No.	221795	Prepared Plates – Pkg. of 20*

BBL™ Modified Thayer-Martin (MTM II) Agar (JEMBEC™)

BS12 CMPH2 MCM9

Cat. No.	221806	Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

T

Thermoacidurans Agar

Intended Use

Thermoacidurans Agar is used for isolating and cultivating *Bacillus coagulans* (*Bacillus thermoacidurans*) from foods.

User Quality Control

Identity Specifications

Difco™ Thermoacidurans Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in purified water upon boiling. Solution is light amber, opalescent.
Prepared Appearance:	Light amber, opalescent.
Reaction of 3.9% Solution at 25°C:	pH 5.0 ± 0.2

Cultural Response

Difco™ Thermoacidurans Agar

Prepare the medium per label directions. Inoculate and incubate at 55 ± 1°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus coagulans	7050	10^2-10^3	Good

Summary and Explanation

Stern et al.[1] described a medium for isolating *B. coagulans* (*B. thermoacidurans*), which causes "flat sour" spoilage in tomato juice and other canned foods. Bacterial growth results in a 0.3-0.5 drop in pH; the ends of the can remain flat. *B. coagulans* is a soil microorganism that can be found in canned tomato products and dairy products. Conditions favorable to multiplication of the organism can result in spoilage of the food product.[2]

Thermoacidurans Agar can also be used to isolate mesophilic spore-forming anaerobes (*Clostridium* spp.) from foods. These microorganisms tolerate high heat, grow in the absence of oxygen and grow over the range of temperatures used in canned and processed foods. They are of primary importance in spoilage of low-acid foods packed in hermetically sealed containers.[2]

Principles of the Procedure

Thermoacidurans Agar contains peptone to provide the carbon and nitrogen for general growth requirements. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Agar is the solidifying agent.

Formula

Difco™ Thermoacidurans Agar

Approximate Formula* Per Liter		
Yeast Extract	5.0	g
Proteose Peptone	5.0	g
Dextrose	5.0	g
Dipotassium Phosphate	4.0	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 39 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[1,2]

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

Microorganisms other than *B. coagulans* may grow on this medium. Perform microscopic examination and biochemical tests to identify to genus and species if necessary.

References

1. Stern, Hegarty and Williams. 1942. Food Research 7:186.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Thermoacidurans Agar

CCAM COMPF

Cat. No. 230310 Dehydrated – 500 g

Thiamine Assay Medium • Thiamine Assay Medium LV

Intended Use

Thiamine Assay Medium is used for determining thiamine concentration by the microbiological assay technique using *Lactobacillus fermentum* ATCC™ 9338.

Thiamine Assay Medium LV is used for determining thiamine concentration by the microbiological assay technique using *Weissella* (*Lactobacillus*) *viridescens* ATCC™ 12706.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of medium are used for this purpose:

1. Maintenance Medium: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Medium: To condition the test culture for immediate use;
3. Assay Medium: To permit quantitation of the vitamin under test. Assay media contain all factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Thiamine Assay Medium is prepared according to the formula by Sarett and Cheldelin.[1] *Lactobacillus fermentum* ATCC 9338 is used as the test organism in the microbiological assay of thiamine (vitamin B_1).

Thiamine Assay Medium LV, patterned after APT medium, was described by Deibel, Evans and Niven[2] for the microbiological assay of thiamine using *Lactobacillus viridescens* ATCC 12706.

Nutritional studies by Evans and Niven[3] on the heterofermentative lactobacilli that cause greening in cured meat products indicated that thiamine was an essential vitamin for growth of these organisms. Deibel, Evans and Niven[4] described APT medium for lactobacilli cultivation. They reported that lactobacilli required at least 10 ng thiamine per mL for growth in contrast to 0.2 to 3 ng per mL for thiamine-requiring streptococci, leuconostocs and staphylococci. Further, they suggested that lactobacilli requiring large amounts of thiamine might be employed in microbiological assay procedures. In 1957,[2] these authors described a medium for the microbiological assay of thiamine using *Lactobacillus* (now *Weissella*) *viridescens* ATCC 12706 as the test organism. This medium is known as Thiamine Assay Medium LV.

Principles of the Procedure

Thiamine Assay Medium and Thiamine Assay Medium LV are free from thiamine, but contain all other nutrients and vitamins essential for the growth of the test organisms. The addition of thiamine in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formulae

Difco™ Thiamine Assay Medium

Approximate Formula* Per Liter		
Thiamine-Free Tryptone	22.0	g
Vitamin Assay Casamino Acids	5.0	g
Dextrose	40.0	g
Sodium Acetate	15.0	g
L-Cystine	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Riboflavin	200.0	µg
Calcium Pantothenate	200.0	µg
Niacin	200.0	µg
Pyridoxine Hydrochloride	200.0	µg
p-Aminobenzoic Acid	200.0	µg
Folic Acid	5.0	µg
Biotin	0.8	µg
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg

Difco™ Thiamine Assay Medium LV

Approximate Formula* Per Liter		
Thiamine-Free Yeast Extract	10.0	g
Thiamine-Free Tryptone	20.0	g
Dextrose	20.0	g
Sodium Citrate	10.0	g
Dipotassium Phosphate	10.0	g
Sodium Chloride	10.0	g
Magnesium Sulfate	1.6	g
Manganese Sulfate	0.28	g
Ferrous Sulfate	0.08	g
Polysorbate 80	2.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care to avoid contamination of media or glassware must be taken in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 100 mL of purified water:
   Difco™ Thiamine Assay Medium - 8.5 g;
   Difco™ Thiamine Assay Medium LV - 8.4 g.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Dispense in 5 mL amounts into tubes, evenly dispensing the precipitate.
4. Add standard or test samples.
5. Adjust the volume to 10 mL with purified water.
6. Autoclave at 121°C for 5 minutes.

User Quality Control

Identity Specifications

Difco™ Thiamine Assay Medium

Dehydrated Appearance:	Beige, homogeneous, tendency to clump.
Solution:	4.25% (single strength) and 8.5% (double strength) solution, soluble in purified water upon boiling 2-3 minutes. 4.25% solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	4.25% solution is light amber, clear, may have a slight precipitate.
Reaction of 4.25% Solution at 25°C:	pH 6.5 ± 0.2

Difco™ Thiamine Assay Medium LV

Dehydrated Appearance:	Beige, homogeneous, tendency to clump.
Solution:	4.2% (single strength) and 8.4% (double strength) solution, soluble in purified water upon boiling 2-3 minutes. 4.2% solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	4.2% solution is light amber, clear, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Difco™ Thiamine Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus fermentum* ATCC™ 9338 when prepared in single strength and supplemented with thiamine. The medium should produce a standard curve when tested using a thiamine hydrochloride reference standard at 0.0 to 0.05 µg per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-18 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Difco™ Thiamine Assay Medium LV

Prepare the medium per label directions. The medium supports the growth of *Weissella viridescens* ATCC™ 12706 when prepared in single strength and supplemented with thiamine. The medium should produce a standard curve when tested using a thiamine hydrochloride reference standard at 0.0 to 25.0 ng per 10 mL. Incubate tubes with caps loosened at 30 ± 2°C for 16-20 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Procedure

Thiamine Assay Medium

Prepare stock cultures of the test organism, *Lactobacillus fermentum* ATCC 9338, by stab inoculation on Lactobacilli Agar AOAC or Micro Assay Culture Agar. After 24-48 hours incubation at 35-37°C, keep the tubes in the refrigerator. Make transfers in triplicate at monthly intervals.

Prepare the inoculum by subculturing a stock culture of the test organism in 10 mL of Lactobacilli Broth AOAC or Micro Inoculum Broth. After 16-18 hours incubation at 35-37°C, centrifuge the cells under aseptic conditions and decant the supernatant liquid. Wash the cells three times with 10 mL sterile 0.85% NaCl. After the third wash, resuspend the cells in 10 mL sterile 0.85% NaCl. Add 0.5 mL of this suspension to 100 mL sterile 0.85% NaCl. Use one drop of the resulting suspension to inoculate the assay tubes.

A standard curve should be run with each assay because conditions of heating and incubation temperature that influence the standard curve readings cannot always be duplicated.

The tubes for the Thiamine Assay Medium standard curve contain 0.0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04 and 0.05 µg of thiamine hydrochloride per 10 mL tube. The most effective assay range for Thiamine Assay Medium is between 0.005 and 0.03 µg thiamine.

Prepare the stock solution of thiamine required for the preparation of the standard curve in Thiamine Assay Medium as follows:

1. Dissolve 0.1 g of thiamine hydrochloride in 1,000 mL of purified water (100 µg/mL).
2. Add 1 mL of the solution in Step 1 to 99 mL purified water (1 µg/mL).
3. Add 1 mL of the solution in Step 2 to 99 mL purified water to give a final concentration of 10 ng (0.010 µg/mL). Use 0.0, 0.5, 1, 1.5, 2, 3, 4 and 5 mL of this final solution per tube. Prepare fresh stock solution daily.

After 20-24 hours incubation at 35-37°C, *L. fermentum* ATCC 9338 is capable of using the pyrimidine and thiazole moieties of the thiamine molecule. It is essential that the growth response be measured turbidimetrically prior to this time. Incubate the tubes at 35-37°C for 16-18 hours, then place in the refrigerator for 15-30 minutes to stop growth. The growth can then be measured by any suitable nephelometric method.

Thiamine Assay Medium LV

Prepare stock cultures of the test organism, *W. viridescens* ATCC 12706, by stab inoculation on APT Agar or Lactobacilli Agar AOAC. After 24-48 hours incubation at 30 ± 2°C, keep the tubes in the refrigerator. Make transfers in triplicate at monthly intervals.

Prepare the inoculum by subculturing a stock culture of the test organism to 10 mL APT Broth or Lactobacilli Broth AOAC. After 16-20 hours incubation at 30 ± 2°C, centrifuge the cells under aseptic conditions and decant the supernatant liquid. Wash the cells three times with 10 mL sterile 0.85% NaCl. After the third wash, resuspend the cells in 10 mL sterile 0.85% NaCl. Add 1 mL of this cell suspension to 100 mL sterile 0.85% NaCl. Use one drop of this suspension to inoculate the assay tubes.

A standard curve should be run with each assay because conditions of heating and incubation temperature that influence the standard curve readings cannot always be duplicated.

The standard curve for Thiamine Assay Medium LV is obtained by using thiamine at levels of 0.0, 1, 2.5, 5, 7.5, 10, 15, 20 and 25 ng of thiamine hydrochloride per 10 mL tube. This is obtained by using 0.0, 0.2, 0.5, 1, 1.5, 2, 3, 4 and 5 mL of the standard solution, which contains 5 ng (0.005 µg) thiamine hydrochloride per mL. The most effective assay range is between 2.5 and 20 ng per tube.

The solution for preparing the standard curve for Thiamine Assay Medium LV may be prepared as follows:

1. Dissolve 50 mg of thiamine hydrochloride in 500 mL purified water (100 µg/mL).
2. Add 1 mL of the solution in Step 1 to 99 mL purified water (1 µg/mL).
3. Add 1 mL of the solution in Step 2 to 199 mL purified water to give a final concentration of 5 ng (0.005 µg) per mL.

Following incubation of *W. viridescens* ATCC 12706 at 30 ± 2°C for 16-20 hours, the growth response is measured turbidimetrically.

Expected Results

Thiamine Assay Medium and Thiamine Assay Medium LV

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average and use the results only if two-thirds of the values do not vary more than ± 10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the microbiological assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements which will not give a satisfactory response.
4. For successful results, all conditions of the assay must be followed exactly.

References

1. Sarett and Cheldelin. 1944. J. Biol. Chem. *155*:153.
2. Deibel, Evans and Niven. 1957. Abstr. A68, p. 28. Bacteriol. Proc. 57th Gen. Meet. Soc. Am. Bacteriologists. 1957.
3. Evans and Niven. 1951. J. Bacteriol. 62:599.
4. Diebel, Evans and Niven. 1955. Abstr. G56, p. 48. Bacteriol. Proc. 55th Gen. Meet. Soc. Am. Bacteriologists. 1955.

Availability

Difco™ Thiamine Assay Medium

Cat. No. 232610 Dehydrated – 100 g*

Difco™ Thiamine Assay Medium LV

Cat. No. 280810 Dehydrated – 100 g*

**Store at 2-8°C.*

Thioglycollate Media
Fluid Thioglycollate Medium • NIH Thioglycollate Broth • Sterility Test Broth • Thioglycollate Medium, Brewer Modified • Fluid Thioglycollate Medium with Beef Extract • Thioglycollate Medium without Dextrose • Thioglycollate Medium (Fluid), without Dextrose or (Eh) Indicator • Thioglycollate Medium without Indicator (135C) • Fluid Thioglycollate Medium, Enriched • Enriched Thioglycollate Medium Thioglycollate Medium with Calcium Carbonate Enriched Thioglycollate Medium with Calcium Carbonate

Intended Use

Fluid Thioglycollate Medium (FTM) is used for the sterility testing of biologics and for the cultivation of anaerobes, aerobes and microaerophiles.

NIH Thioglycollate Broth and Sterility Test Broth (USP Alternative Thioglycollate Medium) may be used for sterility testing instead of FTM.

Thioglycollate Medium, Brewer Modified is used for the cultivation of obligate anaerobes, microaerophiles and facultative organisms.

Fluid Thioglycollate Medium with Beef Extract is used in cultivating microorganisms from normally sterile biological products.

Thioglycollate Medium without Dextrose and Thioglycollate Medium (Fluid), without Dextrose or (Eh) Indicator are used as bases for fermentation studies of anaerobes, as well as for detecting microorganisms in normally sterile materials, especially those containing mercurial preservatives.

Thioglycollate Medium without Indicator (135C) is an enriched general-purpose medium for the recovery of a wide variety of microorganisms, particularly obligate anaerobes, from clinical specimens and other materials.

Fluid Thioglycollate Medium, Enriched and Enriched Thioglycollate Medium are general-purpose media used in qualitative procedures for the cultivation of fastidious, as well as nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical specimens. Enriched Thioglycollate Medium when supplemented with sodium bicarbonate or a marble chip is used to prepare a standardized inoculum by the growth method for antimicrobial susceptibility testing of anaerobic bacteria.

Thioglycollate Medium with Calcium Carbonate and Thioglycollate Medium, Enriched, with Calcium Carbonate are recommended for the maintenance of stock cultures.

Fluid Thioglycollate Medium and NIH Thioglycollate Broth/ Sterility Test Broth meet *United States Pharmacopeia* (*USP*) performance specifications.

Summary and Explanation

Quastel and Stephenson[1] found that the presence of a small amount of a compound containing an –SH group (cysteine, thioglycollic acid, glutathione) permitted "aerobic" growth of *Clostridium sporogenes* in tryptic digest broth.

Falk, Bucca and Simmons[2] pointed out the advantages of using small quantities of agar (0.06-0.25%) in detecting contaminants during sterility testing of biologicals. The value of combining a small amount of agar and a reducing substance was demonstrated by Brewer.[3] Brewer's experiments revealed that in a liquid medium containing 0.05% agar, anaerobes grew equally well in the presence or absence of sodium thioglycollate. Marshall, Gunnish and Luxen[4] reported satisfactory cultivation of anaerobes in Brewer's Thioglycollate Medium in the presence of a mercurial preservative. Nungester, Hood and Warren[5] and Portwood[6] confirmed the neutralization of the bacteriostatic effect of mercurial compounds by sodium thioglycollate. Incorporation of casein peptone was introduced by Vera.[7] Malin and Finn[8] reported the commonly used medium containing thioglycollate is inhibitory to some organisms in the presence of a carbohydrate. In 1941, the National Institutes of Health specified the use of two thioglycollate media in sterility testing, the Brewer Formula and the Linden Formula.[9] The Linden Formula was later referred to as Modified Brewer Thioglycollate Medium in which meat infusion was replaced by plant (soy) peptones.[10]

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Fluid Thioglycollate Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.98% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear.
Prepared Appearance:	Light amber, slightly opalescent, 10% or less of upper layer may be medium pink. After shaking solution becomes pink throughout.
Reaction of 2.98% Solution at 25°C:	pH 7.1 ± 0.2

Difco™ NIH Thioglycollate Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.9% Solution at 25°C:	pH 7.1 ± 0.2

Difco™ Fluid Thioglycollate Medium with Beef Extract

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.47% solution, soluble in purified water upon boiling for 1-2 minutes. When hot, solution is medium amber, clear.
Prepared Appearance:	Medium amber with some opalescence, 10% or less of upper layer may be pink. After shaking solution becomes pink throughout.
Reaction of 3.47% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Thioglycollate Medium without Dextrose

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent, 10% or less of upper layer is green.
Reaction of 2.4% Solution at 25°C:	pH 7.2 ± 0.2

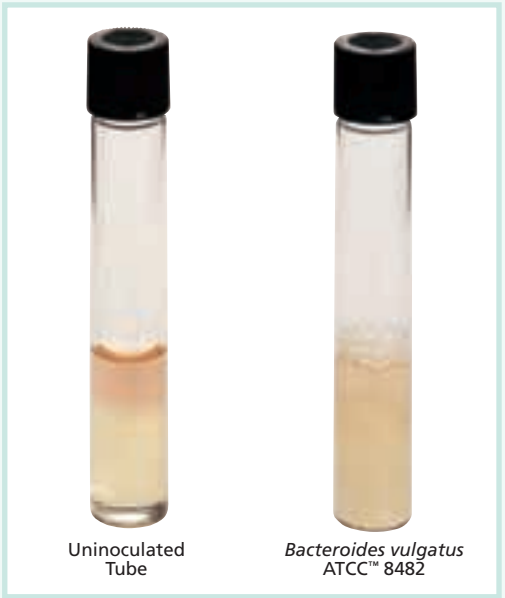
Difco™ Thioglycollate Medium without Dextrose or Indicator

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 2.4% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Thioglycollate Medium without Indicator

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear.
Prepared Appearance:	Light amber, very slightly to slightly opalescent.
Reaction of 2.9% Solution at 25°C:	pH 7.2 ± 0.2

Continued



Uninoculated Tube

Bacteroides vulgatus ATCC™ 8482

Fluid Thioglycollate Medium is recommended in the FDA *Bacteriological Analytical Manual* (BAM)[11] and the *Official Methods of Analysis of AOAC International*[12] for the examination of food, and for determining the phenol coefficient and sporicidal effects of disinfectants. Fluid Thioglycollate Medium is also specified for sterility checks on banked blood.[13] It is one of the media recommended in the *USP* for use in sterility testing of articles purporting to be sterile; these formulations meet the requirements of the *USP* growth promotion test.[14]

NIH Thioglycollate Broth and Sterility Test Broth, which are the USP Alternative Thioglycollate Medium, are Fluid Thioglycollate Medium without the agar or indicator components. They are used for the same sterility test procedures except that anaerobic incubation is recommended rather than aerobic incubation. They also meet the requirements of the *USP* growth promotion test.[14]

Fluid Thioglycollate Medium with Beef Extract is recommended by the Animal and Plant Health Inspection Service, USDA,[15] in the detection of viable bacteria in live vaccines. Thioglycollate Medium without Dextrose and Thioglycollate Medium without Dextrose or Indicator may be used with added carbohydrates for fermentation studies.

Cultural Response

Difco™ Fluid Thioglycollate Medium

Prepare the medium per label directions. Inoculate and incubate at 30-35°C for 18-48 hours (up to 72 hours, if necessary). To test for growth promotion according to the USP/EP, inoculate using organisms marked with (*) and incubate aerobically at 30-35°C for up to 5 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	USP/EP GROWTH
Clostridium novyi	7659	10-10^2	Good	N/A
Clostridium perfringens	13124	10-10^2	Good	N/A
Staphylococcus aureus	25923	10-10^2	Good	N/A
*Bacillus subtilis**	6633	10-10^2	N/A	Growth
*Bacteroides vulgatus**	8482	10-10^2	N/A	Growth
*Clostridium sporogenes**	11437	10-10^2	N/A	Growth
*Clostridium sporogenes**	19404	10-10^2	N/A	Growth
*Kocuria rhizophila**	9341	10-10^2	N/A	Growth
*Pseudomonas aeruginosa**	9027	10-10^2	N/A	Growth
*Staphylococcus aureus**	6538	10-10^2	N/A	Growth

Mercurial Neutralization Test – To perform, add 1% Merthiolate™* to medium, inoculate (10^3 CFU) with *Staphylococcus aureus* ATCC 6538P and *Streptococcus pyogenes* ATCC 19615, and incubate at 30-35°C for 18-48 hours. Recovery of organisms indicates that Merthiolate has been neutralized.

Difco™ NIH Thioglycollate Broth

Prepare the medium per label directions. Inoculate duplicate tubes and incubate at 30-35°C for 18-48 hours (up to 72 hours, if necessary) under anaerobic conditions (tight caps).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides vulgatus	8482	10-10^2	Poor to good
Clostridium sporogenes	11437	10-10^2	Poor to good
Clostridium sporogenes	19404	10-10^2	Poor to good

Mercurial Neutralization Test – To perform, add 1% Merthiolate™ to medium, inoculate (10^3 CFU) with *Staphylococcus aureus* ATCC 6538P and *Streptococcus pyogenes* ATCC 19615, and incubate at 30-35°C for 18-48 hours. Recovery of organisms indicates that Merthiolate has been neutralized.

**Merthiolate is a trademark of Eli Lilly and Company.*

Difco™ Fluid Thioglycollate Medium with Beef Extract

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10-10^2	Good
Bacteroides vulgatus	8482	10-10^2	Good
Candida albicans	10231	10-10^2	Good
Clostridium chauvoei	10092	10-10^2	Good
Clostridium perfringens	13124	10-10^2	Good
Clostridium sporogenes	19404	10-10^2	Good
Kocuria rhizophila	9341	10-10^2	Good

Mercurial Neutralization Test – To perform, add 1% Merthiolate™ to medium, inoculate (10^3 CFU) with *Staphylococcus aureus* ATCC 6538P and *Streptococcus pyogenes* ATCC 19615, and incubate at 30-35°C for 18-48 hours. Recovery of organisms indicates that Merthiolate has been neutralized.

Difco™ Thioglycollate Medium without Dextrose, Thioglycollate Medium without Indicator* or Thioglycollate Medium without Dextrose or Indicator*

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	25285	10-10^2	Poor to fair†
Bacteroides vulgatus	8482	10-10^2	Poor to fair†
Clostridium novyi	7659	10-10^2	Good
Clostridium sporogenes	11437	10-10^2	Good
Staphylococcus aureus	25923	10-10^2	Good

*Mercurial Neutralization Test – To perform, add 1% Merthiolate™ to medium, inoculate (10^3 CFU) with *Staphylococcus aureus* ATCC 6538P and *Streptococcus pyogenes* ATCC 19615, and incubate at 30-35°C for 18-48 hours. Recovery of organisms indicates that Merthiolate has been neutralized.

† Poor to good for Thioglycollate Medium without Indicator.

Continued

Thioglycollate Medium without Indicator (135C) is the medium of choice for diagnostic work because the lack of indicator avoids possible toxicity to organisms.[11] This medium supports a minimal inoculum with early visibility of growth.

When used as an enrichment broth to support plated media, thioglycollate media are often supplemented with hemin and vitamin K_1.[16] Fluid Thioglycollate Medium, Enriched is **BBL**™ Fluid Thioglycollate Medium supplemented with vitamin K_1 and hemin. Enriched Thioglycollate Medium is **BBL** Thioglycollate Medium without Indicator-135C supplemented with vitamin K_1 and hemin. Enriched broth media are recommended for use in the isolation and cultivation of fastidious or slow growing, obligately anaerobic microorganisms present in clinical materials.[17,18] They are also recommended for the isolation and cultivation of a wide variety of aerobic and facultatively anaerobic microorganisms. Enriched Thioglycollate Medium is prepared with an anaerobic head space and is provided in screw-capped tubes in accordance with CDC recommendations.[17] Vitamin K_1 and hemin have been shown to be required by certain anaerobes for growth.[19,20] The addition of calcium carbonate enhances the maintenance of stock cultures by neutralizing acids produced during growth.[16] The Enriched Thioglycollate Medium (Broth) recommended by the CLSI for inoculum preparation for susceptibility tests of anaerobes consists of Enriched Thioglycollate Medium (Thioglycollate Medium without Indicator [135] with 1 µg/mL of Vitamin K_1 and 5 µg/mL of hemin) supplemented with 1 mg/mL of sodium bicarbonate or a marble chip to neutralize acids produced during growth of the test organisms.[21]

Principles of the Procedure

Dextrose, peptone, L-cystine and yeast extract provide the growth factors necessary for bacterial replication. Sodium chloride provides essential ions. Sodium thioglycollate is a reducing agent that prevents the accumulation of peroxides which are lethal to some microorganisms. The L-cystine is also a reducing agent, since it contains sulfhydryl groups which inactivate heavy metal compounds and maintain a low redox potential, thereby supporting anaerobiosis. Methylene blue is

Identity Specifications

BBL™ Fluid Thioglycollate Medium

Dehydrated Appearance: Medium fine to fine, homogeneous, free of extraneous material.

Solution: 2.95% solution, soluble in purified water upon boiling. When hot, solution is pale to light, yellow to tan, clear.

Prepared Appearance: Pale to light, yellow to tan with light pink to rose indicator on top, moderately hazy to hazy.

Reaction of 2.95% Solution at 25˚C: pH 7.1 ± 0.2

BBL™ Sterility Test Broth

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 2.85% solution, soluble in purified water upon boiling. When hot, solution is light to medium, yellow to tan, clear to slightly hazy.

Prepared Appearance: Light to medium, yellow to tan, clear to slightly hazy.

Reaction of 2.85% Solution at 25˚C: pH 7.1 ± 0.2

BBL™ Thioglycollate Medium, Brewer Modified

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 3.85% solution, soluble in purified water upon boiling. When hot, solution is light to medium, yellow to tan, clear.

Prepared Appearance: Light to medium, yellow to tan with medium green indicator color at the top, moderately hazy to hazy.

Reaction of 3.85% Solution at 25˚C: pH 7.2 ± 0.2

BBL™ Thioglycollate Medium, Fluid, without Dextrose or Eh Indicator

Dehydrated Appearance: Fine, homogeneous, free of extraneous material and may contain tan specks.

Solution: 2.4% solution, soluble in purified water upon boiling. When hot, solution is pale to light, yellow to tan, clear.

Prepared Appearance: Pale to light, yellow to tan, slightly to moderately hazy.

Reaction of 2.4% Solution at 25˚C: pH 7.1 ± 0.2

BBL™ Thioglycollate Medium without Indicator – 135C

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 3.0% solution, soluble in purified water upon boiling. When hot, solution is pale to light, tan to yellow, clear.

Prepared Appearance: Pale to light, tan to yellow, moderately hazy to hazy.

Reaction of 3.0% Solution at 25˚C: pH 7.0 ± 0.2

Cultural Response

BBL™ Fluid Thioglycollate Medium

Prepare the medium per label directions. Inoculate and incubate aerobically at 30-35˚C for up to 5 days. To test for growth promotion according to the USP/EP, test with all of the organisms listed.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Bacillus subtilis	6633	$<10^2$	Growth
Bacteroides vulgatus	8482	$<10^2$	Growth
Clostridium sporogenes	11437	$<10^2$	Growth
Clostridium sporogenes	19404	$<10^2$	Growth
Kocuria rhizophila	9341	$<10^2$	Growth
Pseudomonas aeruginosa	9027	$<10^2$	Growth
Staphylococcus aureus	6538	$<10^2$	Growth

BBL™ Sterility Test Broth

Prepare the medium per label directions. Inoculate and incubate at 30-35˚C for 7 days. Incubate organisms marked with (*) anaerobically (with tight caps).

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Bacillus subtilis	6633	$\leq10^3$	Growth
*Bacteroides vulgatus**	8482	$\leq10^2$	Growth
Candida albicans	10231	$\leq10^3$	Growth
*Clostridium sporogenes**	11437	$\leq10^2$	Growth
Kocuria rhizophila	9341	$\leq10^3$	Growth
Streptococcus pneumoniae	6305	$\leq10^3$	Growth

BBL™ Thioglycollate Medium, Brewer Modified

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2˚C for 7 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Bacteroides fragilis	25285	$\leq10^3$	Growth
Staphylococcus aureus	25923	$\leq10^3$	Growth
Streptococcus pyogenes	19615	$\leq10^3$	Growth

BBL™ Thioglycollate Medium, Fluid, without Dextrose or Eh Indicator

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2˚C for 7 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Clostridium sporogenes	11437	$\leq10^3$	Growth
Staphylococcus aureus	25923	$\leq10^3$	Growth

BBL™ Thioglycollate Medium without Indicator – 135C

Prepare the medium per label directions. Inoculate and incubate as indicated below.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TIME/TEMP	RESULT
Bacteroides fragilis	25285	$\leq10^3$	7 days/35 ± 2˚C	Satisfactory
Campylobacter jejuni subsp. *jejuni*	33291	$\leq10^3$	7 days/40-44˚C	Satisfactory
Saccaharomyces cerevisiae	9763	$\leq10^3$	7 days/25 ± 2˚C	Satisfactory
Staphylococcus aureus	25923	$\leq10^3$	7 days/35 ± 2˚C	Satisfactory
Streptococcus pyogenes	19615	$\leq10^3$	7 days/35 ± 2˚C	Satisfactory

an indicator of the level of oxidation/reduction in the medium; increased oxidation raises the Eh, causing the methylene blue indicator to become green. Resazurin is an oxidation-reduction indicator, being pink when oxidized and colorless when reduced. The small amount of agar assists in the maintenance of a low redox potential by stabilizing the medium against convection currents, thereby maintaining anaerobiosis in the lower depths of the medium. The *USP* lists 5.5g/L of dextrose in the formulations for Fluid Thioglycollate Medium and Alternative Thioglycollate Medium; some of the following formulations include the anhydrous form of dextrose (5.0g/L).

Vitamin K_1 is a growth requirement for some strains of *Prevotella melaninogenica*[18] and is reported to enhance the growth of some strains of *Bacteroides* species and gram-positive nonsporeformers.[22] Hemin is the source of the X factor, which stimulates the growth of many microorganisms.

Calcium carbonate neutralizes acids produced during growth, which helps to maintain the viability of fastidious organisms; e.g., pneumococci, gram-negative cocci, *Clostridium perfringens* and other acid-sensitive bacteria.

Dipotassium phosphate is a buffering agent.

Formulae

Difco™ Fluid Thioglycollate Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose	5.5	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Resazurin	1.0	mg

BBL™ Fluid Thioglycollate Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose (anhydrous)	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Resazurin	1.0	mg

Difco™ NIH Thioglycollate Broth

Approximate Formula* Per Liter		
Casitone	15.0	g
Yeast Extract	5.0	g
Dextrose	5.5	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g

BBL™ Sterility Test Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose (anhydrous)	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g

BBL™ Thioglycollate Medium, Brewer Modified

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	17.5	g
Papaic Digest of Soybean Meal	2.5	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
Sodium Thioglycollate	1.0	g
Dipotassium Phosphate	2.0	g
Methylene Blue	2.0	mg
Agar	0.5	g

Difco™ Fluid Thioglycollate Medium with Beef Extract

Approximate Formula* Per Liter		
Beef Extract	5.0	g
Yeast Extract	5.0	g
Pancreatic Digest of Casein	15.0	g
Dextrose	5.5	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Resazurin	1.0	mg

Difco™ Thioglycollate Medium without Dextrose

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.25	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Methylene Blue	2.0	mg

Difco™ Thioglycollate Medium without Dextrose or Indicator

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.25	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g

BBL™ Thioglycollate Medium, Fluid, without Dextrose or Eh Indicator

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	20.0	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g

Difco™ Thioglycollate Medium without Indicator

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.25	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g

BBL™ Thioglycollate Medium without Indicator – 135C

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	17.0	g
Papaic Digest of Soybean Meal	3.0	g
Dextrose	6.0	g
Sodium Chloride	2.5	g
L-Cystine	0.25	g
Sodium Thioglycollate	0.5	g
Agar	0.7	g
Sodium Sulfite	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Fluid Thioglycollate Medium – 29.8 g;
   BBL™ Fluid Thioglycollate Medium – 29.5 g;
   Difco™ NIH Thioglycollate Broth – 29 g;
   BBL™ Sterility Test Broth – 28.5 g;
   BBL™ Thioglycollate Medium, Brewer Modified – 38.5 g;
   Difco™ Fluid Thioglycollate Medium w/Beef Extract – 34.7 g;
   Difco™ Thioglycollate Medium w/o Dextrose – 24 g;
   Difco™ Thioglycollate Medium w/o Dextrose or Indicator – 24 g;
   BBL™ Thioglycollate Medium, Fluid, w/o Dextrose or Eh Indicator – 24 g;
   Difco™ Thioglycollate Medium w/o Indicator – 29 g;
   BBL™ Thioglycollate Medium w/o Indicator – 135C – 30 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Follow label directions specific for each medium.
4. Autoclave at 121˚C for 15 minutes, or as directed on the label.
5. Store fluid thioglycollate media at 15-30˚C. If more than 30% of the medium is pink prior to use, reheat once (100˚C) to drive off absorbed oxygen.
6. Test samples of the finished product for performance using stable, typical control cultures.

Precautions

Do not reheat the media more than once; continued reheating gives rise to toxicity.

Procedure

Follow the procedures outlined in the references and, where applicable, in product package inserts.

Expected Results

After incubation, growth is evidenced by the presence of turbidity compared to an uninoculated control. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in that portion of the broth below the upper oxidized layer.

Limitation of the Procedure[16]

Anaerobes can be overgrown by more rapidly growing facultative organisms. Examine and Gram stain broth if plating medium reveals no growth. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.

References

1. Quastel and Stephenson. 1926. J. Biochem. *20*:1125.
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14. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
15. Federal Register. 1992. Fed. Regist. *21*:113.26.
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18. Chapin. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
19. Gibbons and MacDonald. 1960. J. Bacteriol. *80*:164.
20. Wilkins, Chalgren, Jimeniz-Ulate, Drake and Johnson. 1976. J. Clin. Microbiol. *3*:359.
21. Clinical and Laboratory Standards Institute. 2003. Approved standard: M11-A5. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 5th ed. CLSI, Wayne, Pa.
22. Finegold, Sutter, Attebery and Rosenblatt. 1974. *In* Lennette, Spaulding and Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Fluid Thioglycollate Medium

AOAC BAM BS12 COMPF EP USDA USP

Cat. No.	225640	Dehydrated – 100 g
	225650	Dehydrated – 500 g
	225620	Dehydrated – 2 kg
	225630	Dehydrated – 10 kg

BBL™ Fluid Thioglycollate Medium

AOAC BAM BS12 COMPF EP USDA USP

Cat. No.	211260	Dehydrated – 500 g
	211263	Dehydrated – 5 lb (2.3 kg)
	211264	Dehydrated – 25 lb (11.3 kg)
	221195	Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
	221196	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*
	220888	Prepared Tubes, 20 mL (A Tubes) – Pkg. of 10
	220889	Prepared Tubes, 20 mL (A Tubes) – Ctn. of 100
	299108	Prepared Bottles, 100 mL (serum) – Pkg. of 10
	299417	Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10
	257217	Sterile Pack Bottles (double bagged), 100 mL – Pkg. of 10
	299112	Prepared Bottles, 500 mL (septum screw cap) – Pkg. of 10
	257407	Prepared Bottles, 200 mL (flip off cap and stopper) – Pkg. of 10

Europe

Cat. No.	257408	Prepared Bottles, 300 mL – Pkg. of 10
	257409	Prepared Bottles, 500 mL – Pkg. of 4
	257406	Prepared Bottles, 600 mL – Pkg. of 4
	257370	Prepared Bottles (ETO), 100 mL – Ctn. of 44
	257426	Prepared Tubes (ETO), 18 mL – Ctn. of 60
	257246	Prepared Bottles, 100 mL (flip off cap and stopper) – Pkg. of 25
	257249	Prepared Bottles, 100 mL (125 mL capacity, screw cap) – Pkg. of 25
	257275	Prepared Bottles, 100 mL (150 mL capacity, screw cap) – Pkg. of 25
	257422	Prepared Bottles (wide mouth), 150 mL – Pkg. of 25
	257264	Sterile Pack Bottles (double bagged), 100 mL – Pkg. of 10
	257290	Sterile Pack Bottles (double bagged), 800 mL – Pkg. of 4
	257097	Sterile Pack Bottles (ETO), 100 mL – Ctn. of 44
	257317	Sterile Pack Bottles (wide mouth), 150 mL – Pkg. of 25

Difco™ NIH Thioglycollate Broth (USP Alternative Thioglycollate Medium)

USP

Cat. No. 225710 Dehydrated – 500 g

BBL™ Sterility Test Broth (USP Alternative Thioglycollate Medium)

USP

Cat. No. 211651 Dehydrated – 500 g

BBL™ Thioglycollate Medium, Brewer Modified

Cat. No. 211716 Dehydrated – 500 g

Difco™ Fluid Thioglycollate Medium with Beef Extract

Cat. No. 269720 Dehydrated – 500 g
269710 Dehydrated – 10 kg

Difco™ Thioglycollate Medium without Dextrose

Cat. No. 236310 Dehydrated – 500 g

Difco™ Thioglycollate Medium without Dextrose or Indicator

Cat. No. 243210 Dehydrated – 500 g

BBL™ Thioglycollate Medium, Fluid, without Dextrose or Eh Indicator

Cat. No. 211727 Dehydrated – 500 g
221398 Prepared Tubes (K Tubes) – Ctn. of 100*

Difco™ Thioglycollate Medium without Indicator

Cat. No. 243010 Dehydrated – 500 g

BBL™ Thioglycollate Medium without Indicator – 135C

BS12 CMPH2

Cat. No. 211720 Dehydrated – 500 g
221199 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
221200 Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*
221797 Prepared Tubes, 10 mL (D Tubes) – Pkg. of 10*
221798 Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100*
221047 Prepared Tubes, 20 mL (A Tubes) – Ctn. of 100*

BBL™ Fluid Thioglycollate Medium, Enriched

Cat. No. 297642 Prepared Tubes (K Tubes) – Ctn. of 100*

BBL™ Enriched Thioglycollate Medium

BS12 CLSI CMPH2 MCM9

Cat. No. 221741 Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*
221742 Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100*
221787 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
221788 Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*
297289 Prepared Tubes, 10 mL (D Tubes) – Pkg. of 10*
297292 Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100*

BBL™ Thioglycollate Medium with Calcium Carbonate Chip

Cat. No. 298518 Prepared Tubes (K Tubes) – Ctn. of 100

BBL™ Enriched Thioglycollate Medium with Calcium Carbonate

Cat. No. 297264 Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100*

**Store at 2-8°C.*

Thiol Broth

Intended Use

Thiol Broth is used for cultivating organisms from body fluids and other materials containing penicillin, streptomycin or sulfonamides.

Summary and Explanation

Szawatkowski[1] and Shanson and Barnicoat[2] reported Thiol Broth to be superior in supporting the growth of *Bacteroides* species in blood cultures. Thiol Broth was used to study the optimum incubation period of blood culture broths.[3] Media containing thiol and thioglycollate are recommended for recovery of nutritionally variant streptococci (NVS).[4]

Thiol Broth is cited in the first edition of *Clinical Microbiology Procedures Handbook*[5] as a medium specific for anaerobic bacteria in blood cultures.

Principles of the Procedure

Peptones and yeast extract provide nitrogen, vitamins and amino acids in Thiol Broth. Dextrose is a carbon source. Sodium chloride maintains osmotic balance. Para-aminobenzoic acid is a preservative. Sodium thioglycollate and L-cystine are rich in sulfhydryl (-SH) groups, which neutralize the bacteriostatic and bactericidal effects of penicillin, streptomycin and sulfonamides.

Formula

Difco™ Thiol Broth

Approximate Formula* Per Liter		
Proteose Peptone No.3	10.0	g
Pancreatic Digest of Casein	4.35	g
Gelatin	1.0	g
Yeast Extract	5.0	g
Dextrose	0.2	g
Sodium Chloride	5.0	g
L-Cystine, Disodium	2.4	g
Sodium Thioglycollate	1.0	g
p-Aminobenzoic Acid	0.05	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 29 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on processing and interpretation of blood cultures and other specimens, refer to appropriate references.[5,6]

User Quality Control

Identity Specifications

Difco™ Thiol Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in purified water upon boiling. Solution is very light to light amber, clear to slightly opalescent.
Prepared Appearance:	Very light amber, clear to slightly opalescent.
Reaction of 2.9% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Difco™ Thiol Broth

Prepare the medium per label directions. Test without and with concentrations of 5, 100 and 1,000 units of penicillin and 100, 1,000 and 10,000 µg of streptomycin per 10 mL tube. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY w/o ANTIBIOTICS	RECOVERY w/ANTIBIOTICS
Staphylococcus aureus	25923	10^2-10^3	Good	Good†
Streptococcus pyogenes	19615	10^2-10^3	Good	Good†

†Antibiotic concentrations up to 100 units of penicillin or 1,000 µg of streptomycin.

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

Strict reliance on blood culture bottles containing Thiol Broth is not recommended for aerobic microorganisms. Always use an aerobic medium for optimum isolation of the broad spectrum of microorganisms that can cause bacteremia or septicemia.

References

1. Szawatkowski. 1976. Med. Lab. Sci. *33*:5.
2. Shanson and Barnicoat. 1975. J. Clin. Pathol. *28*:407.
3. Murray. 1985. J. Clin. Microbiol. *21*:481.
4. Donnelly. 1994. Infect. Dis. Alert *6*:109.
5. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
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Availability

Difco™ Thiol Broth

Cat. No. 243420 Dehydrated – 500 g

Tinsdale Agar Base
Tinsdale Enrichment Desiccated

Intended Use

Tinsdale Agar Base is used with Tinsdale Enrichment Desiccated in isolating and differentiating *Corynebacterium diphtheriae*.

Summary and Explanation

Tinsdale Agar Base, supplemented with Tinsdale Enrichment, is employed in the cultural diagnosis of diphtheria. Diphtheria, an acute infectious disease primarily of the upper respiratory tract but occasionally of the skin,[1] is caused by toxigenic strains of *Corynebacterium diphtheriae*. The three biotypes are mitis, intermedius and gravis.[1] The signs and symptoms of the disease are a pharyngeal membrane, sore throat, malaise, headache and nausea.[2] Death can result from respiratory obstruction by the membrane or myocarditis caused by the toxin.[2]

Tinsdale[3] developed a serum-cystine-thiosulfate-tellurite agar medium for the primary isolation and differentiation of *C. diphtheriae*. This formulation distinguished between *C. diphtheriae* and diphtheroids which exhibited similar characteristics. The differential principle is based on the capacity of *C. diphtheriae* to produce a brown or black halo around the colonies.

Billings[4] simplified Tinsdale Basal Medium by using Proteose Peptone No. 3 as a nutrient source. This modification improved the differential qualities and recovery of *C. diphtheriae*. Moore and Parsons[5] confirmed the halo formation of *C. diphtheriae* with one exception; *C. ulcerans* occasionally produced colonies similar to *C. diphtheriae* and required biochemical identification.

Principles of the Procedure

Peptone provides the nitrogen, vitamins, carbon and amino acids in Tinsdale Agar Base. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Tinsdale Enrichment Desiccated contains bovine serum and horse serum, which provide essential growth factors. L-cystine and sodium thiosulfate provide sulfur for H_2S production. Potassium tellurite is a selective agent. The formation of black to brown halos surrounding the colony results from the reduction of potassium tellurite by H_2S to metallic tellurite.

Stabbing the medium with an inoculating needle accentuates darkening of the medium by *C. diphtheriae*.

Formulae

Difco™ Tinsdale Agar Base

Approximate Formula* Per Liter		
Proteose Peptone No. 3	20.0	g
Sodium Chloride	5.0	g
Agar	20.0	g

User Quality Control

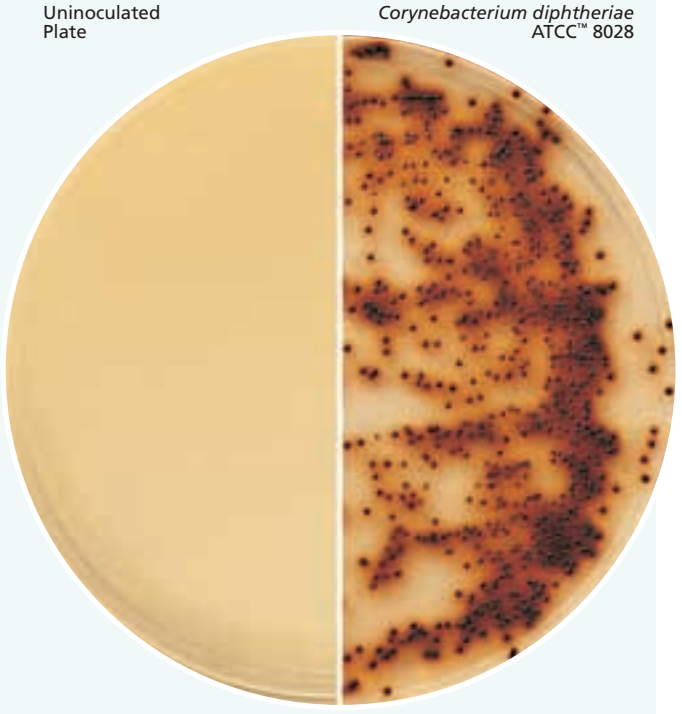
Identity Specifications

Difco™ Tinsdale Agar Base

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent to opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent to opalescent.
Reaction of 4.5% Solution at 25°C:	pH 7.4 ± 0.2

Difco™ Tinsdale Enrichment Desiccated

Desiccated Appearance:	Light to dark tan cake; variations may occur.
Solution:	Soluble in purified water. Solution is light to dark amber, clear to opalescent, may have a slight precipitate.



Cultural Response

Difco™ Tinsdale Agar Base with Tinsdale Enrichment Desiccated

Prepare the medium per label directions. Inoculate to obtain discrete colonies and stab several times using an inoculating needle; incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
Corynebacterium diphtheriae biotype gravis	8028	10^2-10^3	Good	Brown with halos
Corynebacterium diphtheriae biotype mitis	8024	10^2-10^3	Good	Brown with halos
Klebsiella pneumoniae	13883	10^2-10^3	Marked to complete inhibition	–
Streptococcus pyogenes	19615	10^2-10^3	Poor to fair	Brown to black without halos

Difco™ Tinsdale Enrichment Desiccated

Approximate Formula* Per Liter

Bovine Serum	333	mL
Horse Serum	380	mL
L-Cystine	2.0	g
Potassium Tellurite	1.4	g
Sodium Thiosulfate	2.8	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Tinsdale Agar Base

1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense 100 mL amounts into flasks.
4. Autoclave at 121°C for 15 minutes.
5. Aseptically add 15 mL rehydrated Tinsdale Enrichment to each 100 mL at 50-55°C. Mix well.
6. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Tinsdale Enrichment Desiccated

1. Rehydrate with 15 mL sterile purified water.
2. Rotate in an end-over-end motion to dissolve completely.

Procedure

1. For a complete discussion on the collection, isolation and identification of *C. diphtheriae* and other *Corynebacterium* species, refer to the appropriate procedures outlined in the references.[1,2,6]
2. Inoculate plates with the test organisms in a manner to obtain discrete colonies and stab the medium several times with an inoculating needle.
3. Definitive identification of a strain of *C. diphtheriae* as a true pathogen requires demonstration of toxin production.[6]

Expected Results

The appearance of brown-black colored colonies surrounded by brown-black halos is presumptive evidence for *C. diphtheriae*.[1]

Limitations of the Procedure

1. Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C. diphtheriae*.[1]
2. *C. ulcerans*, *C. pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar.[1]

3. Do not read Tinsdale Agar early because several organisms may exhibit slight browning on this medium in 18 hours.[1]
4. Incubation in 5-10% CO_2 retards the development of halos on Tinsdale Agar.[1]
5. On media containing tellurite, diphtheria bacilli are shorter and stain more uniformly; however, granules are less readily observed than when grown on Loeffler's medium.[7]
6. Further biochemical tests may be necessary to distinguish between *C. diphtheriae* and *C. ulcerans* due to similar reactions on this medium.

References

1. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
2. Funke and Bernard. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Tinsdale. 1947. J. Pathol. Bacteriol. *59*:461.
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5. Moore and Parsons. 1958. J. Infect. Dis. *102*:88.
6. Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
7. Bailey and Scott. 1966. Diagnostic microbiology, 2nd ed. The C. V. Mosby Company, St. Louis, Mo.

Availability

Difco™ Tinsdale Agar Base

Cat. No. 278610 Dehydrated – 500 g

Difco™ Tinsdale Enrichment Desiccated

Cat. No. 234210 Tube – 6 × 15 mL*

**Store at 2-8°C.*

Bacto™ Todd Hewitt Broth • Todd Hewitt Broth Todd Hewitt Broth with Gentamicin and Nalidixic Acid

Intended Use

Todd Hewitt Broth is a general-purpose medium, which primarily is used for the cultivation of beta-hemolytic streptococci, especially for serological studies.

Todd Hewitt Broth with Gentamicin and Nalidixic Acid is used for the selective enrichment of group B streptococci (*Streptococcus agalactiae*), especially from genital specimens.

Summary and Explanation

Todd Hewitt broth originally was developed for use in the production of streptococcal hemolysin.[1] The modification of Updyke and Nickle[2] is used for the growth of beta-hemolytic streptococci for use in fluorescent antibody test procedures[3] and for serological typing based on the production of type-specific M protein.[4]

Since its emergence in the 1970s, neonatal group B streptococcal disease has become the major infectious cause of illness and death among newborns. Prior to 1994, an estimated 7,600 episodes of invasive group B streptococcal disease, primarily sepsis and meningitis, occurred in newborns each year in the United States, with approximately 80% of those episodes representing early-onset disease occurring within the first week of life.[5] The disease is spread to newborns through vertical transmission from a mother who carries group B streptococci in her anorectum or genital tract.

The Centers for Disease Control and Prevention (CDC) has published guidelines for screening and use of intrapartum chemoprophylaxis for prevention of neonatal group B streptococcal disease.[6] The use of Todd Hewitt Broth with Gentamicin and Nalidixic Acid (or Lim Broth) is recommended to maximize the likelihood of recovering group B streptococci upon plating on sheep blood agar.

Group B streptococci have also been found in cases of sepsis in nonparturient women and in men and in joint infection, osteomyelitis, urinary tract infection and wound infection. They are associated with endocarditis, pneumonia and pyelonephritis in immunosuppressed patients.[7]

Principles of the Procedure

Todd Hewitt Broth is highly nutritious due to its content of peptones, dextrose and salts. Dextrose stimulates hemolysin production. Sodium phosphate and sodium carbonate provide buffering action to counteract the acidity produced during fermentation of dextrose, thereby protecting the hemolysin from inactivation by the acid.[4]

Selectivity for group B streptococci is obtained by the inclusion of gentamicin and nalidixic acid in the medium. Selective enrichment broths include the advantages of both enrichment and selection by providing conditions conducive to the growth of group B streptococci while inhibiting the growth of contaminants.

Formula

Bacto™ Todd Hewitt Broth

Approximate Formula* Per Liter		
Heart, Infusion from 500 g	3.1	g
Neopeptone	20.0	g
Dextrose	2.0	g
Sodium Chloride	2.0	g
Disodium Phosphate	0.4	g
Sodium Carbonate	2.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 30 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Bacto™ Todd Hewitt Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.0% Solution at 25°C:	pH 7.8 ± 0.2

Cultural Response

Bacto™ Todd Hewitt Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good
Streptococcus pneumoniae	6303	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

Procedure

Incubate throat swabs in loosely-capped tubes of Todd Hewitt Broth at 35 ± 2°C in an aerobic atmosphere with or without added carbon dioxide for 2-5 hours prior to use in fluorescent antibody procedures for the identification of group A streptococci. Incubation may be continued for approximately 24 hours prior to streaking for isolation on blood agar plates. Pure cultures of streptococci may be cultured in Todd Hewitt Broth prior to the preparation of extracts for serological typing.

Consult appropriate references for specific serological test procedures.[2,8]

Incubate tubes of Todd Hewitt Broth with Gentamicin and Nalidixic Acid in an aerobic atmosphere with or without added carbon dioxide. If turbidity is observed, subculture from the broth culture to a sheep blood agar plate; otherwise, incubate an additional 24 hours before discarding.[9]

Expected Results

Growth in broth medium is indicated by the presence of turbidity compared to an uninoculated control.

Subculture to a **Trypticase**™ Soy Agar with 5% Sheep Blood (TSA II) plate and incubate for 18-24 hours, or up to 48 hours if necessary. Identify organisms suggestive of group B streptococci (β- or non-hemolytic, gram-positive and catalase negative). Specific identification may be performed; e.g., using streptococcal grouping sera, the CAMP test or other procedures.

References

1. Todd and Hewitt. 1932. J. Pathol. Bacteriol. *35*:973.
2. Updyke and Nickle. 1954. Appl. Microbiol. *2*:117.
3. Jones, Hebert and Cherry. 1978. Fluorescent antibody techniques and bacterial applications, HEW Publication (CDC) No. 78-8364. Center for Disease Control, Atlanta, Ga.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Federal Register. 1994. Prevention of group B streptococcal disease: a public health perspective. Fed. Regist. *59*:64764.
6. Centers for Disease Control and Prevention. 2002. Morbid. Mortal. Weekly Rep. *51*(No. RR-11): 1.
7. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
8. Facklam and Washington II. 1991. *In* Balows, Hausler, Hermann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
9. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Bacto™ Todd Hewitt Broth

Cat. No.	249240	Dehydrated – 500 g
	249210	Dehydrated – 2 kg
	249220	Dehydrated – 10 kg

BBL™ Todd Hewitt Broth

Cat. No.	297778	Prepared Tubes (K Tubes), 0.5 mL – Pkg. of 10
	221713	Prepared Tubes (K Tubes), 5 mL – Pkg. of 10
	221714	Prepared Tubes (K Tubes), 5 mL – Ctn. of 100

BBL™ Todd Hewitt Broth with Gentamicin and Nalidixic Acid

Cat. No.	299486	Prepared Tubes (K Tubes) – Ctn. of 100*

**Store at 2-8°C.*

T

Tomato Juice Media
Tomato Juice Agar • Tomato Juice Agar Special
Tomato Juice Broth

Intended Use

Tomato Juice Agar is used for cultivating and enumerating *Lactobacillus* species.

Tomato Juice Agar Special is used for cultivating and enumerating lactobacilli and other acidophilic microorganisms from saliva and other specimens.

Tomato Juice Broth is used for cultivating yeasts and other aciduric microorganisms.

Summary and Explanation

In 1925, Mickle and Breed[1] reported the use of tomato juice in culture media used for cultivating lactobacilli. Kulp[2] investigated the use of tomato juice on bacterial development and found that the growth of *L. acidophilus* was enhanced. Tomato Juice Agar, prepared according to Kulp and White's[3] modification, is especially useful in cultivating *L. acidophilus* from clinical specimens and foodstuffs.[4]

User Quality Control

Identity Specifications

Difco™ Tomato Juice Agar

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in purified water upon boiling. Solution is medium to dark amber, very slightly opalescent.
Prepared Appearance:	Medium to dark amber, very slightly opalescent.
Reaction of 5.1% Solution at 25°C:	pH 6.1 ± 0.2

Difco™ Tomato Juice Agar Special

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in purified water upon boiling. Solution is medium to dark amber, slightly opalescent.
Prepared Appearance:	Medium to dark amber, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 5.0 ± 0.2

Difco™ Tomato Juice Broth

Dehydrated Appearance:	Tan, free-flowing, homogeneous and may contain dark particles.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is dark amber, clear.
Prepared Appearance:	Dark amber, clear.
Reaction of 4.1% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Tomato Juice Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus acidophilus	4356	10^2-10^3	Good
Lactobacillus rhamnosus	9595	10^2-10^3	Good
Lactobacillus delbrueckii subsp. *lactis*	4797	10^2-10^3	Good

Difco™ Tomato Juice Agar Special

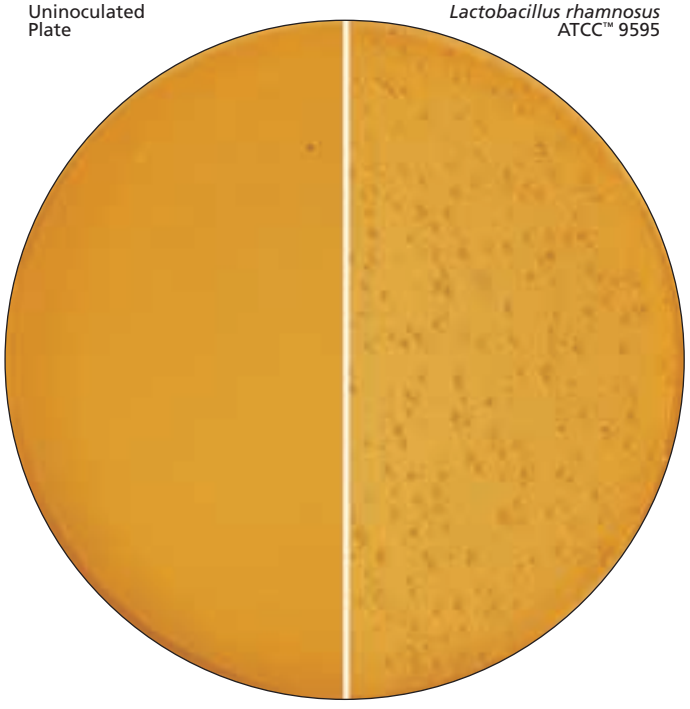
Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours (72 hours if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus acidophilus	4356	10^2-10^3	Good
Lactobacillus rhamnosus	9595	10^2-10^3	Good
Lactobacillus delbrueckii subsp. *lactis*	4797	10^2-10^3	Good

Difco™ Tomato Juice Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus rhamnosus	9595	10^2-10^3	Good
Lactobacillus delbrueckii subsp. *lactis*	4797	10^2-10^3	Good
Saccharomyces cerevisiae	9080	10^2-10^3	Good
Saccharomyces cerevisiae	9763	10^2-10^3	Good



Tomato Juice Agar Special is recommended for the direct plate count of lactobacilli from saliva and for cultivation of other acidophilic microorganisms. The acidic pH of Tomato Juice Agar Special encourages growth of lactobacilli while inhibiting growth of accompanying bacteria. The number of lactobacilli in saliva is an index of a predisposition to dental caries as described by Jay.[5,6] Many dentists use the direct count of lactobacilli for the diagnosis of caries. This medium is more selective for lactobacilli than Tomato Juice Agar.

Tomato Juice Broth is recommended for use in cultivating and isolating yeasts, lactobacilli and other aciduric microorganisms from clinical specimens and foods.

Principles of the Procedure

Tomato Juice Agar and Tomato Juice Agar Special

Tomato juice is a source of carbon, protein and nutrients. Peptone provides a source of nitrogen, amino acids and carbon. Peptonized milk contains lactose as an energy source. Agar is the solidifying agent.

Tomato Juice Broth

Tomato juice is a source of carbon, protein and nutrients. Yeast extract is a source of trace elements, vitamins and amino acids. Dipotassium phosphate and monopotassium phosphate provide buffering capability. Magnesium sulfate, ferrous sulfate and manganese sulfate provide inorganic ions. Sodium chloride is a source of essential ions.

Formulae

Difco™ Tomato Juice Agar

Approximate Formula* Per Liter		
Tomato Juice (from 400 mL)	20.0	g
Peptone	10.0	g
Peptonized Milk	10.0	g
Agar	11.0	g

Difco™ Tomato Juice Agar Special

Approximate Formula* Per Liter		
Tomato Juice (from 400 mL)	20.0	g
Peptone	10.0	g
Peptonized Milk	10.0	g
Agar	20.0	g

Difco™ Tomato Juice Broth

Approximate Formula* Per Liter		
Tomato Juice (from 400 mL)	20.0	g
Yeast Extract	10.0	g
Dextrose	10.0	g
Dipotassium Phosphate	0.5	g
Monopotassium Phosphate	0.5	g
Magnesium Sulfate	0.1	g
Sodium Chloride	0.01	g
Ferrous Sulfate	0.01	g
Manganese Sulfate	0.01	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Equilibrate the medium to room temperature before opening.

1. Suspend the powder in 1 L of purified water:
   Difco™ Tomato Juice Agar – 51 g;
   Difco™ Tomato Juice Agar Special – 60 g;
   Difco™ Tomato Juice Broth – 41 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating Tomato Juice Agar Special, which could cause a softer medium.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Mickle and Breed. 1925. Technical Bulletin 110. N.Y. State Agriculture Exp. Station, Geneva, N.Y.
2. Kulp. 1927. Science 66:512.
3. Kulp and White. 1932. Science 76:17.
4. MacFaddin. 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol 1. Williams & Wilkins, Baltimore, Md.
5. Jay and Gordon (ed). 1938. Bacteriology and immunology of dental caries and dental science and dental art. Lea and Febiger, Philadelphia, Pa.
6. Jay, Pelton and Wisan. 1949. Dentistry in public health. W. B. Saunders Company, Philadelphia, Pa.

Availability

Difco™ Tomato Juice Agar

Cat. No. 211794 Dehydrated – 500 g*

Difco™ Tomato Juice Agar Special

Cat. No. 238910 Dehydrated – 500 g*

Difco™ Tomato Juice Broth

Cat. No. 251720 Dehydrated – 500 g*
251710 Dehydrated – 10 kg*

**Store at 2-8°C.*

T

Transport Media
Transport Medium Amies • Transport Medium (Stuart, Toshach and Patsula) • Cary and Blair Transport Medium

Intended Use

Transport Medium Amies and Transport Medium (Stuart, Toshach and Patsula) are used for collecting, transporting and preserving microbiological specimens.

Cary and Blair Transport Medium is used for collecting, transporting and preserving microbiological specimens, particularly those containing *Vibrio cholerae*.

Summary and Explanation

Transport media are chemically defined, semisolid, nonnutritive, phosphate buffered media that provide a reduced environment. Transport media are formulated to maintain the viability of microorganisms without significant increase in growth.

In 1948, Moffett, Young and Stuart described a medium for transporting gonococcal specimens to the laboratory.[1] Stuart, Toshach and Patsula improved this formulation, introducing what is now known as Stuart's Transport Medium.[2] The ability of Stuart's medium to maintain the viability of gonococci during transport[3,4] led other researchers to explore its use with a variety of specimens. This medium is currently recommended for throat, vaginal and wound samples.

In 1964, Cary and Blair modified Stuart's medium by substituting inorganic phosphates for glycerophosphate and raising the pH to 8.4.[5] The modified medium was effective in maintaining the viability of *Salmonella* and *Shigella*[6,7] in fecal samples. Due to its high pH, Cary and Blair Transport Medium is also effective in maintaining the viability of *Vibrio* cultures for up to four weeks.[8] Cary and Blair Transport Medium is currently recommended for fecal and rectal samples.

Amies[9] confirmed Cary and Blair's observations that an inorganic salt buffer was superior to the glycerophosphate. He further modified the formulation by using a balanced salt solution containing inorganic phosphate buffer, omitting the methylene blue and adding charcoal. This modified medium yielded a higher percentage of positive cultures than the transport medium of Stuart. Transport Medium Amies is recommended for throat, vaginal and wound samples. Amies media are especially suited for specimens containing *Neisseria gonorrhoeae*.

Principles of the Procedure

In the formulations, potassium chloride, calcium chloride, magnesium chloride and sodium chloride provide essential ions that help maintain osmotic balance while controlling permeability of bacterial cells. Monopotassium phosphate and disodium phosphate provide buffering capabilities. Sodium thioglycollate suppresses oxidative changes and provides a reduced environment. Sodium glycerophosphate is a buffer for use with calcium chloride. Methylene blue is a colorimetric pH indicator of the oxidation-reduction state. Charcoal neutralizes fatty acids that are toxic to microorganisms. Agar makes the media semi-solid.

Formulae

Difco™ Transport Medium Amies

Approximate Formula* Per Liter		
Sodium Chloride	3.0	g
Potassium Chloride	0.2	g
Calcium Chloride	0.1	g
Magnesium Chloride	0.1	g
Monopotassium Phosphate	0.2	g
Disodium Phosphate	1.15	g
Sodium Thioglycollate	1.0	g
Charcoal	10.0	g
Agar	4.0	g

BBL™ Transport Medium (Stuart, Toshach and Patsula)

Approximate Formula* Per Liter		
Sodium Thioglycollate	1.0	g
Sodium Glycerophosphate	10.0	g
Calcium Chloride	0.1	g
Methylene Blue	2.0	mg
Agar	3.0	g

BBL™ Cary and Blair Transport Medium

Approximate Formula* Per Liter		
Sodium Thioglycollate	1.5	g
Disodium Phosphate	1.1	g
Sodium Chloride	5.0	g
Agar	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Transport Medium Amies

1. Suspend 20 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense into 6-8 mL screw-cap vials to within 5 mm of the top. Cap tightly.
4. Autoclave at 121°C for 15 minutes.
5. Retighten caps, if necessary. Invert vials just prior to solidification to uniformly distribute the charcoal.
6. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Transport Medium (Stuart, Toshach and Patsula)

1. Suspend 14.1 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in small screw-capped bottles or vials, filling them almost to capacity. Leave only enough space to permit acceptance of a small swab without overflow when in use.
6. Autoclave at 121°C for 10 minutes or steam for 1 hour. After autoclaving, tighten caps immediately.
7. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Cary and Blair Transport Medium

1. Suspend 12.6 g of the powder in 991 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Cool to 50°C and add 9 mL of 1% aqueous calcium chloride.
4. Adjust the pH to approximately 8.4, if necessary.
5. Dispense in 7 mL amounts in 9 mL screw-capped test tubes.
6. Steam for 15 minutes. Cool. Tighten caps.
7. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Obtain specimen with sterile swab. Insert specimen swab(s) into the upper third of the medium in the transport container.
2. Cut with sterile scissors or break-off the protruding portion of the swab stick. Tightly screw the lid on the bottle or vial.
3. Label the bottle or vial and send to the laboratory with minimum delay. Specimens may be refrigerated until ready for shipment.
4. Submit to laboratory within 24 hours for culture and analysis.

Expected Results

Survival of bacteria in a transport medium depends on many factors including the type and concentration of bacteria in the specimen, the formulation of the transport medium, the temperature and duration of transport and inoculation to appropriate culture media within 24 hours.

Optimal growth and typical morphology can only be expected following direct inoculation and appropriate cultivation.

User Quality Control

Identity Specifications

Difco™ Transport Medium Amies

Dehydrated Appearance:	Black, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in purified water upon boiling. Solution is black, opaque.
Prepared Appearance:	Black, opaque, semi-solid.
Reaction of 2.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Transport Medium Amies

Prepare the medium per label directions. Inoculate sterile swabs with suspensions of test organisms containing 10^3-10^4 CFU/0.1 mL. Place swabs in the medium and incubate at room temperature for 18-24 hours. Remove swabs, streak on prepared chocolate agar plates and incubate appropriately. All cultures should be viable.

ORGANISM	ATCC™
Bacteroides fragilis	25285
Haemophilus influenzae Type b	10211
Neisseria gonorrhoeae	43069
Neisseria meningitidis Group B	13090
Streptococcus pneumoniae	6305
Streptococcus pyogenes Group A	19615

Identity Specifications

BBL™ Transport Medium (Stuart, Toshach and Patsula)

Dehydrated Appearance:	Slightly moist, granular, softly clumped, free of extraneous material, may contain minute to small white particles.
Solution:	1.41% solution, soluble in purified water upon boiling. Solution is pale, yellow with light blue-green top, clear to slightly hazy.
Prepared Appearance:	Pale, yellow with light blue-green top, clear to slightly hazy.
Reaction of 1.41% Solution at 25°C:	pH 7.3 ± 0.2

BBL™ Cary and Blair Transport Medium

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	12.6 g/991 mL, soluble in purified water upon boiling. Solution is light to medium, gray, hazy to cloudy.
Prepared Appearance:	Light to medium, gray, hazy to cloudy.
Reaction of 12.6 g/991 mL Solution at 25°C:	pH 8.0 ± 0.5

Cultural Response

BBL™ Transport Medium (Stuart, Toshach and Patsula)

Prepare the medium per label directions. Inoculate charcoal impregnated sterile swabs with heavy suspensions of the test organisms. Place in the medium and incubate at 25 ± 2°C for 66-72 hours. Remove swabs, streak on TSA with 5% sheep blood plates or Chocolate II agar plates for (*) organisms and incubate plates at 35 ± 2°C for 18-24 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	RECOVERY
*Haemophilus influenzae**	10211	Good
*Neisseria gonorrhoeae**	19424	Good
Streptococcus pneumoniae	6305	Good

BBL™ Cary and Blair Transport Medium

Prepare the medium per label directions. Inoculate sterile swabs with heavy suspensions of the test organisms. Place in the medium and incubate at 25 ± 2°C for 18-24 hours. Remove swabs, streak on TSA with 5% sheep blood plates or Chocolate II agar plates for (*) organisms and incubate plates at 35 ± 2°C for 18-24 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	RECOVERY
*Haemophilus influenzae**	10211	Good
*Neisseria gonorrhoeae**	19424	Good
Shigella flexneri	9199	Good
Streptococcus pneumoniae	6305	Good

Limitations of the Procedure

1. Specimens taken from transport media will not exhibit the optimal or comparative growth as expected from direct inoculation and cultivation. These media do, however, provide an adequate degree of preservation for those specimens which cannot be forwarded immediately to the laboratory for prompt evaluation.
2. Viability of cells will diminish over time and some degree of multiplication or growth of contaminants can occur during prolonged periods of transit. This is particularly true of fecal specimens that contain substantial numbers of coliform organisms.
3. The condition of the specimen received by the laboratory for culture is a significant variable in recovery and final identification of the suspect pathogen. An unsatisfactory specimen (overgrown by contaminants, containing nonviable organisms, or having the number of pathogens greatly diminished) can lead to erroneous or inconclusive results.
4. For transport of specimens that may contain *N. gonorrhoeae*, the use of a selective medium, such as JEMBEC™ or Gono-Pak systems, should also be considered.

References

1. Moffett, Young and Stuart. 1948. Br. Med. J. 2:421.
2. Stuart, Toshach and Patsula. 1954. Can. J. Public Health *45*:73.
3. Stuart. 1946. Glasgow M. J. 27:131.
4. Stuart. 1959. Public Health Reports *74*:431.
5. Cary and Blair. 1964. J. Bacteriol. *88*:96.
6. Cary, Matthew, Fusillo and Harkins. 1965. Am. J. Clin. Path. *43*:294.
7. Neuman, Benenson, Hubster and Thi Nhu Tuan. 1971. N. Am. J. Clin. Path. *57*:33.
8. Kelly, Hickman-Brenner and Farmer. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed.. American Society for Microbiology, Washington D.C.
9. Amies. 1967. Can. J. Public Health *58*:296.

Availability

Difco™ Transport Medium Amies

Cat. No. 212225 Dehydrated – 500 g

BBL™ Transport Medium (Stuart, Toshach and Patsula)

Cat. No. 211743 Dehydrated – 500 g*

BBL™ Cary and Blair Transport Medium

CCAM

Cat. No. 211102 Dehydrated – 500 g*

**Store at 2-8°C.*

T

Trichophyton Agars 1 – 7

Intended Use

Trichophyton Agars are differential media used in the presumptive identification of *Trichophyton* species based on nutritional requirements.

Summary and Explanation

Members of the genus *Trichophyton* have specific nutritional requirements that are essential for definitive identification.[1-3] Georg and Camp devised a set of chemically-defined media for differentiation and identification of *Trichophyton* isolates based on specific vitamin and amino acid requirements.[4] These requirements are determined by comparing growth in a basal medium (Trichophyton Agar 1 or 6) with the amount of growth obtained by providing a specific nutrient. Trichophyton Agar 2, 3 and 4 are used with medium 1 to determine whether an isolate requires inositol, thiamine or both. Trichophyton Agar 5, equivalent to Trichophyton Agar 1 with added nicotinic acid (2 mg/L), is used with medium 1 to determine the requirement for nicotinic acid, and medium 7 is used with medium 6 to determine the requirement for histidine.

Principles of the Procedure

Nutritional requirements are determined by inoculating a control medium and a medium enriched with a specific vitamin or amino acid with *Trichophyton* isolates that have been presumptively identified by gross colony characteristics and microscopic morphology.[1-6] Moderate to heavy growth in the vitamin- or amino acid-enriched medium compared to little or no growth in the basal medium indicates that the isolate requires that nutrient.

User Quality Control

Identity Specifications

Difco™ Trichophyton Agars 1, 2, 3, 4, 6 or 7

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	5.9% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 5.9% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Trichophyton Agars 1, 2 or 3

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC™	RECOVERY AGARS 1 & 2	RECOVERY AGAR 3
Trichophyton concentricum	9358	Good	Good
Trichophyton schoenleinii	4822	Good	Good
Trichophyton verrucosum	34470	None to poor	Good

Difco™ Trichophyton Agar 4

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC™	RECOVERY
Trichophyton rubrum	28188	Good
Trichophyton verrucosum	34470	Poor
Trichophyton violaceum	8376	Good

Difco™ Trichophyton Agars 6 or 7

Prepare Trichophyton Agar 6 per label directions, plain and with 0.03 g/L L-Histidine HCl. Prepare Trichophyton Agar 7 per label directions. Inoculate with fresh cultures and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC™	RECOVERY AGAR 6 PLAIN	RECOVERY AGAR 6 WITH L-HISTIDINE HCL	RECOVERY AGAR 7
Microsporum gallinae	22243	Good	Good	Good
Trichophyton megninii	12106	Poor	Good	Good

Uninoculated Tube

Trichophyton schoenleinii ATCC™ 4822

Formulae

Difco™ Trichtophyton Agar 1

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	2.5	g
Dextrose	40.0	g
Monopotassium Phosphate	1.8	g
Magnesium Sulfate	0.1	g
Agar	15.0	g

Difco™ Trichtophyton Agar 2

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	2.5	g
Dextrose	40.0	g
Monopotassium Phosphate	1.8	g
Magnesium Sulfate	0.1	g
Agar	15.0	g
Inositol	50.0	mg

Difco™ Trichtophyton Agar 3

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	2.5	g
Dextrose	40.0	g
Monopotassium Phosphate	1.8	g
Magnesium Sulfate	0.1	g
Agar	15.0	g
Inositol	50.0	mg
Thiamine HCl	200.0	µg

Difco™ Trichtophyton Agar 4

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	2.5	g
Dextrose	40.0	g
Monopotassium Phosphate	1.8	g
Magnesium Sulfate	0.1	g
Agar	15.0	g
Thiamine HCl	200.0	µg

Difco™ Trichtophyton Agar 6

Approximate Formula* Per Liter		
Ammonium Nitrate	1.5	g
Dextrose	40.0	g
Monopotassium Phosphate	1.8	g
Magnesium Sulfate	0.1	g
Agar	15.0	g

Difco™ Trichtophyton Agar 7

Approximate Formula* Per Liter		
Ammonium Nitrate	1.5	g
Histidine HCl	30.0	mg
Dextrose	40.0	g
Monopotassium Phosphate	1.8	g
Magnesium Sulfate	0.1	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Trichophyton Agars 1, 2, 3, 4, 6 and 7

1. Suspend 59 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 12 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Using a sterile inoculating loop or needle, remove a small amount of colony growth from the isolation medium and streak the agar surface. A small inoculum should be used to prevent carry-over of essential nutrients from the isolation medium.

Incubate medium at room temperature for up to 2 weeks.

Expected Results

Record the amount of growth using + to indicate a trace of submerged growth to 4+ to indicate maximum growth. Consult appropriate texts for information needed for interpretation of the results.[1,2]

Limitations of the Procedure

1. It is important that pure cultures from a medium that is not vitamin enriched, such as Sabouraud Dextrose Agar or another general-purpose fungal medium, be used for the inoculum.
2. If cultures are contaminated with bacteria, the cultures should be grown on a fungal medium containing antibiotics for several generations to eliminate the bacteria. Many bacteria synthesize vitamins and may invalidate the test results.
3. When inoculating Trichophyton Agars, take care not to carry-over growth substances from primary cultures to the tube media used in the differential tests. Inocula transferred to the nutrition tubes should be very small.

References

1. Roberts. 1985. *In* Washington (ed.), Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
2. Weitzman, Rosenthal and Silva-Hutner. 1988. Superficial and cutaneous infections caused by molds: dermatomycoses. *In* Wentworth (ed.), Diagnostic procedures for mycotic and parasitic infections, 7th ed. American Public Health Association, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
4. Georg and Camp. 1957. J. Bacteriol. *74*:113.
5. Haley, Transdel and Coyle. 1980. Cumitech 11, Practical methods for culture and identification of fungi in the clinical mycology laboratory. Coord. ed., Sherris. American Society for Microbiology, Washington, D.C.
6. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Trichophyton Agar 1
Cat. No. 287710 Dehydrated – 500 g

BBL™ Trichophyton Agar 1
Cat. No. 296243 Prepared Slants (C Tubes) – Pkg of 10*

Difco™ Trichophyton Agar 2
Cat. No. 287410 Dehydrated – 500 g

BBL™ Trichophyton Agar 2
Cat. No. 296244 Prepared Slants (C Tubes) – Pkg. of 10*

Difco™ Trichophyton Agar 3
Cat. No. 296510 Dehydrated – 500 g

BBL™ Trichophyton Agar 3
Cat. No. 296245 Prepared Slants (C Tubes) – Pkg. of 10*

Difco™ Trichophyton Agar 4
Cat. No. 219710 Dehydrated – 500 g

BBL™ Trichophyton Agar 4
Cat. No. 296246 Prepared Slants (C Tubes) – Pkg. of 10*

BBL™ Trichophyton Agar 5
Cat. No. 297498 Prepared Slants (C Tubes) – Pkg. of 10*

Difco™ Trichophyton Agar 6
Cat. No. 252410 Dehydrated – 500 g

BBL™ Trichophyton Agar 6
Cat. No. 297499 Prepared Slants (C Tubes) – Pkg. of 10*

Difco™ Trichophyton Agar 7
Cat. No. 295510 Dehydrated – 500 g

BBL™ Trichophyton Agar 7
Cat. No. 297500 Prepared Slants (C Tubes) – Pkg. of 10*

**Store at 2-8°C.*

Trichosel™ Broth, Modified

Intended Use

Trichosel Broth, Modified is used for the isolation and cultivation of *Trichomonas* species.

Summary and Explanation

Trichosel Broth, Modified is a modification of the Simplified **Trypticase**™ Serum (STS) Medium of Kupferberg et al. for the cultivation of *Trichomonas* spp.[1] The classical formula has been modified by the addition of beef extract and horse serum and an increased amount of yeast extract to improve performance. Chloramphenicol, a relatively stable antibiotic, replaces the penicillin and streptomycin recommended for addition to the STS base.

Principles of the Procedure

Trichosel Broth, Modified contains casein peptone, cysteine, beef extract and yeast extract as sources of amino acids, nitrogen, sulfur, carbon, vitamins and trace ingredients. Maltose is an energy source for the metabolism of microorganisms including *Trichomonas* spp. Chloramphenicol is a broad spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria. Horse serum is added to provide growth factors required by *Trichomonas*.

User Quality Control

Identity Specifications

BBL™ Trichosel Broth, Modified

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	23.1 g soluble in 950 mL purified water upon boiling. Solution is light to medium, yellow to tan, with blue-green to green ring at top, hazy.
Prepared Appearance:	Light to medium, yellow to tan, with blue-green to green ring at top, hazy.
Reaction of 23.1 g/950 mL Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

BBL™ Trichosel Broth, Modified

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 5 days.

ORGANISM	ATCC™	RECOVERY
Candida albicans	10231	Good
Staphylococcus aureus	25923	Partial to complete inhibition
Trichomonas vaginalis	30001	Good; microscopic examination reveals typical morphology and motility

Formula

BBL™ Trichosel Broth, Modified

Approximate Formula* Per Liter

Pancreatic Digest of Casein	12.0	g
Beef Extract	2.0	g
Yeast Extract	5.0	g
L-Cysteine HCl	1.0	g
Maltose	2.0	g
Agar	1.0	g
Chloramphenicol	0.1	g
Methylene Blue	3.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 23.1 g of the powder in 950 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 118°C for 15 minutes. Immediately tighten caps.
4. Cool and aseptically add 5% horse serum.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate specimens suspected of containing *Trichomonas* organisms into the broth medium using swabs containing the specimen or by alternative methods, as appropriate. Incubate tubes at 35 ± 2°C in an aerobic atmosphere. After 48 hours and 5 days of incubation, prepare a wet mount from the broth and examine microscopically under low power for the presence of flagellate protozoans.

Expected Results

If growth of trichomonads has occurred, organisms of typical morphology will be seen in the broth medium when an aliquot is examined microscopically using low power magnification.

Reference

1. Kupferberg, Johnson and Sprince. 1948. Proc. Soc. Exp. Biol. Med. 67:304.

Availability

BBL™ Trichosel™ Broth, Modified
Cat. No. 211747 Dehydrated – 500 g

BBL™ Trichosel™ Broth, Modified, with 5% Horse Serum
Cat. No. 298323 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*

Triple Sugar Iron Agar • TSI Agar

Intended Use

Triple Sugar Iron Agar (TSI Agar) is used for the differentiation of gram-negative enteric bacilli based on carbohydrate fermentation and the production of hydrogen sulfide.

Summary and Explanation

TSI Agar is used for the determination of carbohydrate fermentation and hydrogen sulfide production in the identification of gram-negative bacilli.[1,2]

Hajna developed the formulation for TSI Agar by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar.[3] The addition of sucrose increased the sensitivity of the medium by facilitating the detection of sucrose-fermenting bacilli, as well as lactose and/or dextrose fermenters.

Carbohydrate fermentation is detected by the presence of gas and a visible color change (from red to yellow) of the pH indicator, phenol red. The production of hydrogen sulfide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube.

Principles of the Procedure

TSI Agar contains three sugars (dextrose, lactose and sucrose), phenol red for detecting carbohydrate fermentation and ferrous ammonium sulfate for detection of hydrogen sulfide production (indicated by blackening in the butt of the tube).

Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow. To facilitate the detection of organisms that only ferment dextrose, the dextrose concentration is one-tenth the concentration of lactose or sucrose. The small amount of acid produced in the slant of the tube during dextrose fermentation oxidizes rapidly, causing the medium to remain red or revert to an alkaline pH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube because it is under lower oxygen tension.

After depletion of the limited dextrose, organisms able to do so will begin to utilize the lactose or sucrose.[2]

To enhance the alkaline condition of the slant, free exchange of air must be permitted by closing the tube cap loosely. If the tube is tightly closed, an acid reaction (caused solely by dextrose fermentation) will also involve the slant.

User Quality Control

Identity Specifications

Difco™ Triple Sugar Iron Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is red, very slightly opalescent, may contain up to a small amount of dark brown precipitate.
Prepared Appearance:	Red, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Triple Sugar Iron Agar

Prepare the medium per label directions. Inoculate with fresh cultures by the stab and streak method and incubate with caps loosened at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	RECOVERY	SLANT	BUTT	GAS	H_2S
Escherichia coli	25922	Good	A	A	+	–
Pseudomonas aeruginosa	9027	Good	K	K	–	–
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	Good	K	A	+	+
Shigella flexneri	12022	Good	K	A	–	–

A = Acid K = Alkaline

Formula

Difco™ Triple Sugar Iron Agar

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Yeast Extract	3.0	g
Pancreatic Digest of Casein	15.0	g
Proteose Peptone No. 3	5.0	g
Dextrose	1.0	g
Lactose	10.0	g
Sucrose	10.0	g
Ferrous Sulfate	0.2	g
Sodium Chloride	5.0	g
Sodium Thiosulfate	0.3	g
Agar	12.0	g
Phenol Red	24.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 65 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense into tubes and autoclave at 121°C for 15 minutes.
4. Cool in a slanted position so that deep butts are formed.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To inoculate, carefully touch only the center of an isolated colony on an enteric plated medium with a cool, sterile needle, stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant. Several colonies from each primary plate should be studied separately, since mixed infections may occur.

Incubate with caps loosened at 35°C and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulfide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

Expected Results

Compare reactions produced by the unknown isolate with those produced by the known control organisms.

Carbohydrate fermentation is indicated by a yellow coloration of the medium. If the medium in the butt of the tube becomes yellow (acidic), but the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose (glucose).

A yellow (acidic) color in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose.

A red (alkaline) color in the slant and butt indicates that the organism being tested is a nonfermenter.

Hydrogen sulfide production results in a black precipitate in the butt of the tube.

Gas production is indicated by splitting and cracking of the medium.

For final identification, perform biochemical tests and other identification procedures with a pure culture of the organism. Consult appropriate references for further information.[4-6]

Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Salmonella* Enteritidis ATCC™ 13076 | *Shigella flexneri* ATCC™ 12022

Limitations of the Procedure

1. Hydrogen sulfide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton[7] showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader[8] found that not all H_2S-positive *Salmonella* are positive on TSI.
2. Sucrose is added to TSI to eliminate some sucrose-fermenting lactose-nonfermenters such as *Proteus* and *Citrobacter* spp.[1]
3. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.
4. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production.[1]
5. A pure culture is essential when inoculating Triple Sugar Iron Agar. If inoculated with a mixed culture, irregular observations may occur.
6. Tubes should be incubated with caps loosened. This allows a free exchange of air, which is necessary to enhance the alkaline condition on the slant.[1]

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Hajna. 1945. J. Bacteriol. *49*:516.
4. Ewing. 1985. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
7. Bulmash and Fulton. 1964. J. Bacteriol. *88*:1813.
8. Padron and Dockstader. 1972. Appl. Microbiol. *23*:1107.

Availability

Difco™ Triple Sugar Iron Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP ISO MCM9 SMD SMWW USDA

Cat. No. 226540 Dehydrated – 500 g

BBL™ TSI Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP ISO MCM9 SMD SMWW USDA

Cat. No. 221038 Prepared Slants – Pkg. of 10*
221039 Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

Tryptic Soy Agar • Trypticase™ Soy Agar (Soybean-Casein Digest Agar)

Intended Use

Tryptic (**Trypticase**) Soy Agar (TSA) is used for the isolation and cultivation of nonfastidious and fastidious microorganisms. It is not the medium of choice for anaerobes.

The 150 × 15 mm-style plates of **Trypticase** Soy Agar are convenient for use with **Taxo**™ factor strips in the isolation and differentiation of *Haemophilus* species.

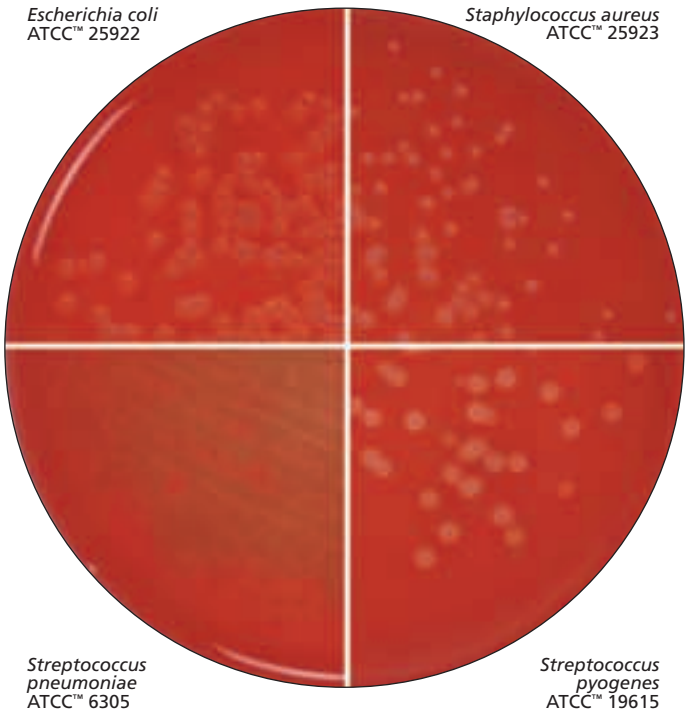
Sterile Pack and Isolator Pack plates are useful for monitoring surfaces and air in clean rooms, Isolator Systems and other environmentally-controlled areas when sterility of the medium is of importance.

Hycheck™ hygiene contact slides are used for assessing the microbiological contamination of surfaces and fluids.

Tryptic (Trypticase) Soy Agar meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

The nutritional composition of TSA has made it a popular medium for many years. It is the medium specified as Soybean-Casein Digest Agar Medium in General Chapter <61> of the *USP* when performing enumerations tests for nonsterile pharmaceutical products.[1] The medium is used in *USP* Growth Promotion testing and when testing the suitability of counting methods in the presence of product. TSA has a multitude of uses in the clinical laboratory including maintenance of stock cultures, plate counting, isolation of microorganisms from a variety of specimen types and as a base for media containing blood.[4-7] It is also recommended for use in industrial applications when testing water and wastewater,[8] food,[9-14] dairy products,[15] and cosmetics.[10,16]



Since TSA does not contain the X and V growth factors, it can conveniently be used in determining the requirements for these growth factors by isolates of *Haemophilus* by the addition of X, V and XV Factor Strips to inoculated TSA plates.[5] The 150 mm plate provides a larger surface area for inoculation, making the "satellite" growth around the strips easier to read.

With the Sterile Pack and Isolator Pack plates, the entire double-wrapped (Sterile Pack) or triple-wrapped (Isolator Pack) product is subjected to a sterilizing dose of gamma radiation, so that the contents inside the outer package(s) are sterile.[17] This allows the inner package to be aseptically removed without introducing contaminants. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent true recovery and not pre-existing medium contaminants. A third rolled sterile bag is included as a transport device. Isolator Pack plates have been validated to protect the medium from vaporized hydrogen peroxide when used in an Isolator System.

The **Hycheck** hygiene contact slide is a double-sided paddle containing two agar surfaces for immersing into fluids or sampling surfaces. There are three slides containing TSA along with another medium: D/E Neutralizing Agar; Violet Red Bile Glucose Agar; or Rose Bengal Chloramphenicol Agar. A fourth slide contains TSA with 0.01% TTC and Rose Bengal Chloramphenicol Agar.

Principles of the Procedure

The combination of casein and soy peptones in TSA renders the medium highly nutritious by supplying organic nitrogen, particularly amino acids and longer-chained peptides. The sodium chloride maintains osmotic equilibrium. Agar is the solidifying agent.

Haemophilus species may be differentiated by their requirements for X and V factors. Paper strips impregnated with these factors are placed on the surface of the medium after inoculation with the test organism. Following incubation, a zone of growth around the strip indicates a requirement for the factor(s).

Formulae

Difco™ Tryptic Soy Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Papaic Digest of Soybean	5.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

BBL™ Trypticase™ Soy Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Papaic Digest of Soybean	5.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Tryptic Soy Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Plain – Light amber, slightly opalescent. With 5% sheep blood – Bright red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Tryptic Soy Agar

Prepare the medium per label directions, without (plain) and with 5% sheep blood (SB). Inoculate and incubate at 35 ± 2°C with 5-10% CO_2 for 18-48 hours. Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY W/SB	HEMOLYSIS
Escherichia coli	25922	30-300	Good	Good	Beta
Neisseria meningitidis	13090	30-300	Good	Good	None
Staphylococcus aureus	25923	30-300	Good	Good	Beta
Streptococcus pneumoniae	6305	30-300	Good	Good	Alpha
Streptococcus pyogenes	19615	30-300	Good	Good	Beta
*Aspergillus brasiliensis (niger)**	16404	<100	Growth	N/A	N/A
*Bacillus subtillis**	6633	<100	Growth	N/A	N/A
*Candida albicans**	10231	<100	Growth	N/A	N/A
*Escherichia coli**	8739	<100	Growth	N/A	N/A
*Pseudomonas aeruginosa**	9027	<100	Growth	N/A	N/A
Salmonella enterica subsp. *enterica* serotype Typhimurium*	14028	<100	Growth	N/A	N/A
*Staphylococcus aureus**	6538	<100	Growth	N/A	N/A

CAMP Test medium with 5% sheep blood – Perform using S. aureus ATCC 33862, Streptococcus sp. Group B ATCC 12386 (positive) and S. pyogenes ATCC 19615 (negative).

Continued

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.
4. For preparation of blood plates, add 5-10% sterile, defibrinated blood to the sterile agar which has been cooled to 45-50°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[4-7]

For water, food, dairy or cosmetic samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[8-16]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using Tryptic/**Trypticase** Soy Agar.[4-7]

For water, food, dairy or cosmetic samples, refer to appropriate standard references for details on test methods using Tryptic/**Trypticase** Soy Agar.[8-16]

For pharmaceutical samples, refer to *USP* General Chapter <61> for details on the examination of nonsterile products and performing microbial enumeration tests using Tryptic/**Trypticase** Soy Agar.[1]

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2. Incubate plates at 35 ± 2°C for 18-24 hours.

Trypticase™ Soy Agar (150 mm plates) for *Haemophilus*

The initial specimens should be inoculated onto Chocolate II Agar or another suitable medium and incubated for 18-24 hours in an aerobic atmosphere supplemented with carbon dioxide. Choose one or two well-isolated colonies that resemble *Haemophilus* species and perform a Gram stain to confirm that the isolate is a gram-negative rod or coccobacillus. Suspend 1-2 colonies in 5 mL sterile, purified water or **Trypticase** Soy Broth and vortex to mix. Dip a swab in the suspension and inoculate the entire surface of the plate with the swab. With sterile forceps, place a **Taxo** X factor strip, a V factor strip and a XV strip on the plate, at least 20 mm apart. Incubate plates at 35 ± 2°C for 24 hours in an aerobic atmosphere supplemented with carbon dioxide.

Identity Specifications

BBL™ Trypticase™ Soy Agar

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 4.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.

Prepared Appearance: Plain – Light to medium, yellow to tan, clear to slightly hazy.

With 5% sheep blood – Bright red, opaque.

Reaction of 4.0% Solution at 25°C: pH 7.3 ± 0.2

BBL™ Trypticase™ Soy Agar (prepared bottle)

Appearance: Light to medium tan yellow, clear to trace hazy.

Reaction at 25°C: pH 7.3 ± 0.2

BBL™ Trypticase™ Soy Agar (prepared plate)

Appearance: Light to medium tan yellow, hazy.

Reaction at 25°C: pH 7.3 ± 0.2

BBL™ Trypticase™ Soy Agar (prepared Sterile Pack plate)

Appearance: Light to medium tan yellow, clear to trace hazy.

Reaction at 25°C: pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Agar

Prepare the medium per label directions, without (plain) and with 5% sheep blood (SB). Inoculate and incubate at 35 ± 2°C for 48 hours (incubate *S. pneumoniae* and *S. pyogenes* with 3-5% CO_2). Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY W/SB	HEMOLYSIS
Candida albicans	10231	10^3-10^4	N/A	Good	None
Pseudomonas aeruginosa	10145	10^3-10^4	Good	N/A	N/A
Shigella flexneri	12022	10^3-10^4	Good	N/A	N/A
Staphylococcus aureus	25923	10^3-10^4	Good	N/A	N/A
Streptococcus pneumoniae	6305	10^3-10^4	Good	Good	Alpha
Streptococcus pyogenes	19615	10^3-10^4	Good	Good	Beta
*Aspergillus brasiliensis (niger)**	16404	<100	Growth	N/A	N/A
*Bacillus subtillis**	6633	<100	Growth	N/A	N/A
*Candida albicans**	10231	<100	Growth	N/A	N/A
*Escherichia coli**	8739	<100	Growth	N/A	N/A
*Pseudomonas aeruginosa**	9027	<100	Growth	N/A	N/A
Salmonella enterica subsp. *enterica* serotype Typhimurium*	14028	<100	Growth	N/A	N/A
*Staphylococcus aureus**	6538	<100	Growth	N/A	N/A

CAMP Test medium with 5% sheep blood – Perform using S. aureus *ATCC 25923,* Streptococcus *sp. Group B ATCC 12386 (positive) and* S. pyogenes *ATCC 19615 (negative).*

BBL™ Trypticase™ Soy Agar (prepared bottle)

Inoculate and incubate at 35-37°C for 48 hours with 3-5% CO_2 (supplemented with sheep blood). Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Streptococcus pneumoniae	6305	10^4-10^5	Good
Streptococcus pyogenes	19615	10^4-10^5	Good
*Aspergillus brasiliensis (niger)**	16404	<100	Growth
*Bacillus subtillis**	6633	<100	Growth
*Candida albicans**	10231	<100	Growth
*Pseudomonas aeruginosa**	9027	<100	Growth
*Staphylococcus aureus**	6538	<100	Growth

BBL™ Trypticase™ Soy Agar (prepared plate)

Inoculate and incubate at 35 ± 2°C for 48 hours (incubate *S. pyogenes* with 3-5% CO_2). Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans).*

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Shigella flexneri	12022	50-100	Good
Staphylococcus aureus	25923	50-100	Good
Streptococcus pyogenes	19615	50-100	Good
*Aspergillus brasiliensis (niger)**	16404	<100	Growth
*Bacillus subtillis**	6633	<100	Growth
*Candida albicans**	10231	<100	Growth
*Pseudomonas aeruginosa**	9027	<100	Growth
*Staphylococcus aureus**	6538	<100	Growth

Inoculate *Haemophilus* strains with a 1:10 dilution from a broth culture and incubate at 35 ± 2°C with 3-5% CO_2 for 24 hours.

ORGANISM	ATCC™	TAXO X	TAXO V	TAXO XV
Haemophilus influenzae	9334	–	–	+
Haemophilus parahemolyticus	10014	–	+	+
Haemophilus parainfluenzae	9796	–	+	+

BBL™ Trypticase™ Soy Agar (prepared Sterile Pack plate)

Inoculate and incubate at 30-35°C for up to 3 days (incubate *A. brasiliensis* at 20-25°C for up to 7 days). Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10-100	Good
Escherichia coli	8739	10-100	Good
Kocuria rhizophila	9341	10-100	Good
Staphylococcus epidermidis	12228	10-100	Good
*Aspergillus brasiliensis (niger)**	16404	10-100	Growth
*Bacillus subtillis**	6633	10-100	Growth
*Candida albicans**	10231	10-100	Growth
*Pseudomonas aeruginosa**	9027	10-100	Growth
*Staphylococcus aureus**	6538	10-100	Growth

T

Expected Results

After incubation, it is desirable to have isolated colonies of organisms from the original sample. Subculture colonies of interest so that positive identification can be made by means of biochemical and/or serological testing.[5-7,18] Consult appropriate texts for the growth patterns produced by the various strains of *Haemophilus*.[5-7]

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Availability

Difco™ Tryptic Soy Agar (Soybean-Casein Digest Agar)

AOAC BAM CCAM COMPF EP EPA ISO JP SMD SMWW USDA USP

Cat. No.	236940	Dehydrated – 100 g†
	236950	Dehydrated – 500 g†
	236920	Dehydrated – 2 kg†
	236930	Dehydrated – 10 kg†

Europe

Cat. No.	256665	Prepared Bottles, 100 mL – Pkg. of 10
	257295	Prepared Plates – Ctn. of 100*

BBL™ Trypticase™ Soy Agar (Soybean-Casein Digest Agar)

AOAC BAM CCAM COMPF EP EPA ISO JP SMD SMWW USDA USP

Cat. No.	211043	Dehydrated – 500 g†
	211046	Dehydrated – 5 lb (2.3 kg)†
	211047	Dehydrated – 25 lb (11.3 kg)†

United States and Canada

Cat. No.	221185	Prepared Plates – Pkg. of 20*†
	221283	Prepared Plates – Ctn. of 100*†
	221803	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*
	221082	Prepared Pour Tubes, 20 mL – Pkg. of 10
	221086	Prepared Tubes (K Tubes) – Pkg. of 10†
	221087	Prepared Tubes (K Tubes) – Ctn. of 100†
	299099	Prepared Bottles, 500 mL – Pkg. of 10†

Europe

Cat. No.	254051	Prepared Plates – Pkg. of 20*
	254086	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251167	Prepared Plates (5 × 4) – Pkg. of 20*
	251185	Prepared Plates – Pkg. of 20*
	251260	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*
	251812	Prepared Plates (60 × 15 mm-style) – Ctn. of 240*

BBL™ Trypticase™ Soy Agar, Sterile Pack

EP JP USP

United States and Canada

Cat. No.	221236	Prepared Settling Plates – Pkg. of 10*
	222205	Prepared Settling Plates – Ctn. of 100*†
	221237	Prepared Settling Plates (150 × 15 mm-style) – Pkg. of 5*
	222206	Prepared Settling Plates (150 × 15 mm-style) – Ctn. of 45*

Europe

Cat. No.	257285	Prepared Plates (150 × 15 mm-style) – Pkg. of 5*
	257284	Prepared Plates (150 × 15 mm-style, triple bagged) – Ctn. of 30*
	254954	Prepared Plates – Pkg. of 10*
	254956	Prepared Plates – Ctn. of 100*
	257076	Prepared Plates (Deep fill) – Pkg. of 10*
	257077	Prepared Plates (Deep fill) – Ctn. of 100*

BBL™ Trypticase™ Soy Agar, Isolator Pack

United States and Canada

Cat. No.	292651	Prepared Plates – Pkg. of 10*
	292652	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	257080	Prepared Plates – Pkg. of 10*
	257081	Prepared Plates – Ctn. of 100*
	257375	Prepared Plates (Deep fill) – Ctn. of 100*
	257427	Prepared **RODAC**™ SL Plates – Ctn. of 100*
	257373	Prepared Plates (150 × 15 mm-style) – Pkg. of 5*
	257377	Prepared Plates (150 × 15 mm-style) – Ctn. of 30*
	257376	Prepared Plates – Pkg. of 10*
	257374	Prepared Plates – Ctn. of 100*

Difco™ Hycheck™ Hygiene Contact Slides

Cat. No.	290002	Tryptic Soy Agar//D/E Neutralizing Agar– Box of 10 slides*
	290003	Tryptic Soy Agar//Violet Red Bile Glucose Agar – Box of 10 slides*
	290006	Tryptic Soy Agar//Rose Bengal Chloramphenicol Agar – Box of 10 slides*
	290007	Tryptic Soy Agar with 0.01% TTC//Rose Bengal Chloramphenicol Agar – Box of 10 slides*

**Store at 2-8°C.*
†QC testing performed according to USP/EP/JP performance specifications.

Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar) • Trypticase™ Soy Agar with Lecithin and Polysorbate 80 • Trypticase™ Soy Agar with Penicillinase • Trypticase™ Soy Agar with Lecithin, Polysorbate 80 and Penicillinase

Intended Use

These media are recommended for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Prepared plates are provided for environmental monitoring. Sterile Pack and Isolator Pack **RODAC**™ prepared plates are particularly useful for monitoring surfaces in clean rooms, Isolator Systems and other environmentally-controlled areas and are also recommended for use in air sampling equipment such as the Surface Air System. **Finger Dab**™ Sterile Pack and Isolator Pack plates are intended for sampling gloved hands.

Summary and Explanation

These media may be employed to establish and monitor cleaning techniques and schedules.[1-4] Collection of "samples" from identical areas before and after treatment with disinfectant yields data useful in evaluating cleaning procedures in environmental sanitation. Tryptic (**Trypticase**) Soy Agar with Lecithin and Polysorbate 80 is recommended for the Aerobic Plate Count (Microbial Limit Test) for water-miscible cosmetic products containing preservatives.[5]

RODAC (Replicate Organism Detection and Counting) and contact plates are used in a wide variety of surface sampling programs and may be employed to establish and monitor cleaning techniques and schedules.[1-4,6] The presence and number of microorganisms on a flat impervious surface is determined by the appearance of colonies on the surface of the medium following application to the test surface and incubation.[7,8] The **RODAC** plate has a marked grid to facilitate counting organisms. The **RODAC** SL (Secure Lid) has three lugs on the base, providing a tight fit between lid and base to reduce accidental contamination.

The 100 × 15 mm and the 150 × 15 mm style plates can be used for active and passive air sampling. These plates are also designed for personnel monitoring of finger tips (**Finger Dab**).

Principles of the Procedure

Casein and soy peptones are a source of nutrients required for the replication of microorganisms. Sodium chloride maintains osmotic equilibrium. Lecithin and polysorbate 80, two commonly used neutralizers, are reported to inactivate residual disinfectants when the sample is being collected.[7] Lecithin is incorporated to neutralize quaternary ammonium compounds and polysorbate 80 is used to neutralize substituted phenolic disinfectants.[9-12] Agar is the solidifying agent.

Trypticase Soy Agar with Penicillinase and **Trypticase** Soy Agar with Lecithin, Polysorbate 80 and Penicillinase contain 50 mL/L of penicillinase, which inactivates antibiotics such as penicillins and cephalosporins.

With the Sterile Pack and Isolator Pack plates, the entire double-wrapped (Sterile Pack) or triple-wrapped (Isolator Pack) product is subjected to a sterilizing dose of gamma radiation, so that the contents inside the outer package(s) are sterile.[13] This allows the inner package to be aseptically removed without introducing contaminants. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent true recovery and not pre-existing medium contaminants. A third rolled sterile bag is included as a transport device. Isolator Pack plates have been validated to protect the medium from vaporized hydrogen peroxide when used in an Isolator System.

Formulae

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Soy Peptone	5.0	g
Sodium Chloride	5.0	g
Lecithin	0.7	g
Polysorbate 80	5.0	g
Agar	15.0	g

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Papaic Digest of Soybean Meal	5.0	g
Sodium Chloride	5.0	g
Lecithin	0.7	g
Polysorbate 80	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45.7 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to approximately 45°C.
4. In **RODAC** plates, use 16.5-17.5 mL per plate.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Dehydrated Appearance:	Beige, free-flowing, homogeneous, may appear moist.
Solution:	4.57% solution, soluble in purified water upon boiling with frequent gentle swirling. When hot, solution is medium amber, slightly opalescent with a resuspendable precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent, may have a precipitate.
Reaction of 4.57% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Prepare the medium per label directions. Test the medium in parallel with Plate Count Agar, using the pour plate method. Apply disks impregnated with varying dilutions of a quaternary ammonium compound to the medium surface. Incubate plates at 35 ± 2°C for 40-48 hours and inspect for zones of inhibition.

ORGANISM	ATCC™	INOCULUM CFU	GROWTH*
Escherichia coli	11229	10^2-10^3	Smaller zone of inhibition of growth compared to Plate Count Agar
Staphylococcus aureus	6538P	10^2-10^3	Smaller zone of inhibition of growth compared to Plate Count Agar

**Interpretation: The smaller zones of inhibition indicate neutralization of the quaternary ammonium compound by the medium.*

Identity Specifications

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

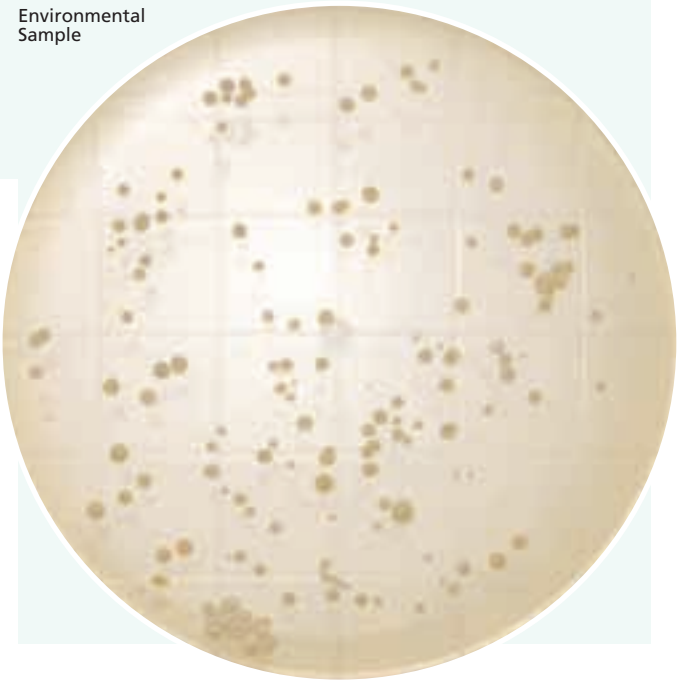
Dehydrated Appearance:	Medium fine, softly lumped powder, free of extraneous material. NOTE: The dehydrated medium has a characteristic "brown sugar" appearance and may seem moist.
Solution:	4.57% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, slightly to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, slightly to moderately hazy.
Reaction of 4.57% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 2 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Pseudomonas aeruginosa	10145	10^3-10^4	Good
Staphylococcus aureus	25923	10^3-10^4	Good



Procedure

100 × 15 mm and 150 × 15 mm-Style Plates

1. If specimen is being cultured from a swab, roll the swab directly on the medium surface.
2. Incubate all plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.
3. When incubation has been completed, count the colonies.

RODAC™/Contact Plates

Selected surfaces are sampled by firmly pressing the agar medium against the test area. Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample. Do not move plate laterally; this spreads contaminants over the agar surface making resolution of colonies difficult. Slightly curved surfaces may be sampled with a rolling motion.

Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.

Grid method:

1. Subdivide surface (floor or wall) into 36 equal squares per 100 square feet of area by striking five equidistant dividing lines from each of the two adjacent sides.
2. These dividing lines intersect at twenty-five points.
3. Number these intersections consecutively in a serpentine configuration.

4. Use red numerals for odd numbers, black numerals for even numbers.
5. Omit number 13 which falls in the center of the total area.
6. Sample odd points at one sampling period, even points at the next sampling period.
7. For areas greater than 100 square feet, extend grid to include entire area.
8. For areas smaller than 25 square feet, divide the areas into twenty-five equal squares (sixteen intersections). Sample eight even-numbered or odd-numbered intersections at each sampling period.
9. For areas between 25 and 100 square feet, divide into 36 equal squares as in #1.
10. Mark plates with intersection numbers.

Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

Expected Results

Because interpretations are relative, each laboratory should establish its own values for what constitutes a clean area.

Count all developing colonies. Spreading colonies should be counted as one but care should be taken to observe other distinct colonies intermingled in the growth around the plate periphery or along a hair line. These should also be counted as one colony, as should bi-colored colonies and halo type spreaders.

It is generally agreed that 200 colonies is the approximate maximum that can be counted on contact plates.

Colony counts may be recorded by:

1. Simply keeping individual counts.
2. Number of viable particles per square foot (agar area is 3.97 square inches).
3. Means and standard deviations.

Subculture colonies of interest so that positive identification can be made by means of biochemical and/or serological testing.

Limitation of the Procedure

The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

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Availability

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

CCAM

Cat. No.	255320	Dehydrated – 500 g*
	255310	Dehydrated – 2 kg*

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

CCAM

Cat. No.	211764	Dehydrated – 500 g*
	212263	Dehydrated – 5 lb (2.3 kg)*

United States and Canada

Cat. No.	221943	Prepared Plates (Double Bag) – Ctn. of 100*
	221945	Contact Plates (Double Bag) – Pkg. of 20*
	221288	Prepared **RODAC**™ Plates – Pkg. of 10*
	221287	Prepared **RODAC**™ Plates – Ctn. of 100*
	222242	Prepared **RODAC**™ SL Plates – Pkg. of 20*
	222249	Prepared **RODAC**™ SL Plates – Ctn. of 100*
	221961	Sterile Pack Contact Plates – Pkg. of 10*
	222208	Sterile Pack Contact Plates – Ctn. of 100*
	221238	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	222207	Sterile Pack **RODAC**™ Plates – Ctn. of 100*
	222248	Sterile Pack **RODAC**™ SL Plates – Pkg. of 10*
	222247	Sterile Pack **RODAC**™ SL Plates – Ctn. of 100*
	292335	Isolator Pack **RODAC**™ Plates – Ctn. of 100*
	222252	Isolator Pack **RODAC**™ SL Plates – Pkg. of 10*
	222253	Isolator Pack **RODAC**™ SL Plates – Ctn. of 100*
	292271	Sterile Pack **Finger Dab**™ Plates – Ctn. of 100*
	292648	Isolator Pack **Finger Dab**™ Plates – Pkg. of 10*
	292649	Isolator Pack **Finger Dab**™ Plates – Ctn. of 100*
	292650	Isolator Pack **Finger Dab**™ Plates (150 × 15 mm-style) – Pkg. of 5*

Europe

Cat. No.	254038	Contact Plates – Pkg. of 33*
	254542	Contact Plates – Pkg. of 220*
	257383	Isolator Pack Plates – Pkg. of 10*
	257384	Isolator Pack Plates – Ctn. of 100*
	257379	Isolator Pack Plates (HF) – Ctn. of 100*
	257380	Isolator Pack **RODAC**™ Plates – Pkg. of 10*
	257381	Isolator Pack **RODAC**™ Plates – Ctn. of 100*
	257378	Isolator Pack **RODAC**™ SL Plates – Pkg. of 10*
	257382	Isolator Pack **RODAC**™ SL Plates – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with Penicillinase

Cat. No.	221839	Sterile Pack Plates – Pkg. of 10*
	221837	Sterile Pack Plates (150 × 15 mm-style) – Pkg. of 5*

BBL™ Trypticase™ Soy Agar with Lecithin, Polysorbate 80 and Pencillinase

United States and Canada

Cat. No.	221987	Contact Plates – Pkg. of 10*
	221234	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	222246	Sterile Pack **RODAC**™ SL Plates – Pkg. of 10*

Europe

Cat. No.	257400	Sterile Pack **RODAC**™ Plates – Ctn. of 100*
	257421	Isolator Pack **RODAC**™ SL Plates – Pkg. of 10*
	257455	Sterile Pack Plates – Ctn. of 100*
	257403	Isolator Pack Plates – Ctn. of 100*

**Store at 2-8°C.*

TSA Blood Agars
Tryptic Soy Blood Agar Base No. 2 • Tryptic Soy Blood Agar Base EH • Trypticase™ Soy Agar, Modified (TSA II) Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) Trypticase™ Soy Agar with 10% Sheep Blood (TSA II) Trypticase™ Soy Agar with 5% Horse Blood (TSA II) Trypticase™ Soy Agar with 5% Rabbit Blood (TSA II)

Intended Use

Tryptic Soy Blood Agar Base No. 2, Tryptic Soy Blood Agar Base EH and **Trypticase** Soy Agar, Modified (TSA II) supplemented with blood are used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

Summary and Explanation

The nutritional composition of TSA (Tryptic Soy Agar/**Trypticase** Soy Agar) has made it a popular medium, both unsupplemented and as a base for media containing blood. **Trypticase** Soy Agar, Modified (TSA II) is an improved version of the original TSA formulation for use with animal blood supplements. With 5 or 10% sheep blood, it is extensively used for the recovery and cultivation of fastidious microbial species and for the determination of hemolytic reactions that are important differentiating characteristics for bacteria, especially *Streptococcus* species. Some investigators prefer the use of rabbit or horse blood, but **Trypticase** Soy Agar with 5% Horse Blood is not recommended for use with throat cultures.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH (enhanced hemolysis) are additional options when hemolytic reactions are important. Tryptic Soy Blood Agar Base No. 2 provides clear hemolytic reactions with group A streptococci, while Tryptic Soy Blood Agar Base EH provides dramatic, improved hemolysis.

Blood agar base media are specified in standard methods for food testing.[1,2]

Principles of the Procedure

The combination of casein and soy peptones in **Trypticase** Soy Agar, Modified (TSA II) renders the medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium. Agar is the solidifying agent.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH are similar in composition to TSA II. These formulations utilize the peptones Tryptone H and Tryptone H Plus to enhance hemolysin production while minimizing antagonism or loss in activity of streptococcal hemolysins.

Defibrinated sheep blood is the most widely used blood for enriching agar base media.[3] Hemolytic reactions of streptococci are proper and growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

Trypticase Soy Agar with 5% Sheep Blood (TSA II) prepared plates provide excellent growth and beta hemolysis by *Streptococcus pyogenes* (Lancefield group A) and also provide excellent growth and appropriate hemolytic reactions with other fastidious organisms. This medium is suitable for performing the CAMP test for the presumptive identification of group B streptococci (*S. agalactiae*), and for use with low concentration (0.04 unit) bacitracin discs (**Taxo**™ A) for presumptive identification of group A streptococci (*S. pyogenes*).

The CAMP test is based on the formation of a zone of synergistic hemolysis at the junction of perpendicular streak inocula of *Staphylococcus aureus* and group B streptococci. The reaction is caused by the sphingomyelinase (beta-toxin) of *S. aureus* reacting with sphingomyelin in the sheep erythrocyte membrane to produce ceramide. A non-enzymatic protein (CAMP protein), produced by *S. agalactiae*, binds to the ceramide and leads to disorganization of the lipid bilayer of the sheep erythrocyte membrane resulting in complete lysis.[4]

Trypticase Soy Agar with 10% Sheep Blood (TSA II) prepared plates are provided for those laboratories preferring the increased blood content. This medium is not recommended for performance of the CAMP test. Additionally, the increased blood content can make hemolytic reactions less distinct and more difficult to read.

Trypticase Soy Agar with 5% Horse Blood (TSA II) prepared plates supply both the X and V factors which are growth requirements for certain organisms; e.g., *Haemophilus influenzae*. Sheep and human blood are not suitable for this purpose because they contain enzymes that inactivate the nicotinamide adenine dinucleotide (NAD), which is the V factor.[5]

Defibrinated horse blood may give hemolytic reactions different from sheep blood.[3] Some streptococci (e.g., group D) give hemolytic reactions on horse blood, but not on sheep blood and

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Tryptic Soy Blood Agar Base No. 2 or Tryptic Soy Blood Agar Base EH

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent.
Prepared Appearance:	Plain – Light to medium amber, clear to slightly opalescent. With 5% sheep blood – Bright cherry red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

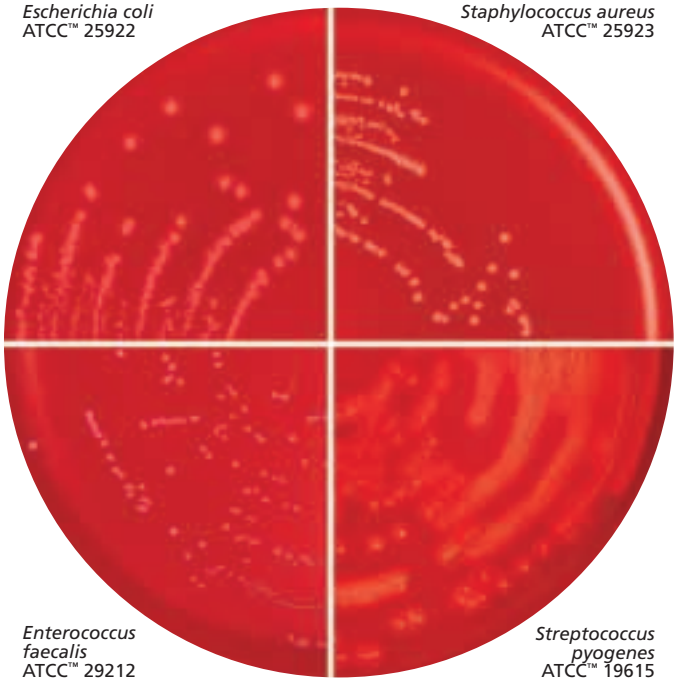
Difco™ Tryptic Soy Blood Agar Base No. 2* or Tryptic Soy Blood Agar Base EH

Prepare the medium per label directions with 5% sheep blood. Inoculate and incubate at 35 ± 2°C for 18-48 hours under 5-10% CO_2.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS TSA NO. 2	HEMOLYSIS TSA EH
Escherichia coli	25922	10^2-10^3	Good	Beta	Beta
Neisseria meningitidis	13090	10^2-10^3	Good	None	None
Staphylococcus aureus	25923	10^2-10^3	Good	Beta	Beta
Streptococcus pneumoniae	6305	10^2-10^3	Good	Alpha	Alpha
Streptococcus pyogenes	19615	10^2-10^3	Good	Beta	Beta

**CAMP Test – Perform using* S. aureus *ATCC 25923,* Streptococcus *sp. group B ATCC 12386 (positive) and* S. pyogenes *ATCC 19615 (negative).*

TSA II with 5% Sheep Blood



Identity Specifications

BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Plain – Light to medium, yellow to tan, clear to slightly hazy. With 5% sheep blood – Bright red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Prepare the medium per label directions with 5% sheep blood. Inoculate and incubate at 35 ± 2°C for 18-48 hours (incubate streptococci with 3-5% CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
Candida albicans	10231	10^3-10^4	Good	None
Escherichia coli	25922	10^3-10^4	Good, within 24 hours	Beta
Listeria monocytogenes	19115	10^3-10^4	Good	Beta (+/-)
Shigella dysenteriae	9361	10^3-10^4	Good	None
Staphylococcus aureus	25923	10^3-10^4	Good, within 24 hours	Beta
Staphylococcus aureus	6538P	10^3-10^4	Good	Beta
Streptococcus pneumoniae	6305	10^3-10^4	Good, within 24 hours	Alpha
Streptococcus pyogenes	19615	10^3-10^4	Good, within 24 hours	Beta
Streptococcus pyogenes	49117	10^3-10^4	Good	Beta

CAMP Test – Perform using S. aureus *ATCC 25923,* Streptococcus *sp. group B ATCC 12386 (positive) and* S. pyogenes *ATCC 19615 (negative).*

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may be mistakenly reported as group A. If a hemolytic reaction is obtained, the organisms should be tested with a **Taxo** A disc and grouped serologically or tested by the fluorescent method.[6] Beta-hemolytic streptococci and *Haemophilus hemolyticus* may be differentiated by performing a Gram stain on a smear prepared from the colony.[7]

Defibrinated rabbit blood is also used for enriching agar-based media.[8] Hemolytic reactions on **Trypticase** Soy Agar with 5% Rabbit Blood (TSA II) prepared plates are similar to those on sheep blood. However, rabbit blood does not inhibit *Haemophilus haemolyticus*, a bacterium inhibited by sheep blood that produces colonies indistinguishable from those of beta-hemolytic streptococci.

Formulae

Difco™ Tryptic Soy Blood Agar Base No. 2

Approximate Formula* Per Liter		
Tryptone H	15.0	g
Soytone	5.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

Difco™ Tryptic Soy Blood Agar Base EH

Approximate Formula* Per Liter		
Tryptone H Plus	15.0	g
Soytone	5.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	14.5	g
Papaic Digest of Soybean Meal	5.0	g
Sodium Chloride	5.0	g
Agar	14.0	g
Growth Factors	1.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.
4. For preparation of blood plates, add 5-10% sterile, defibrinated blood to sterile agar which has been cooled to 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. After streaking, stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.[9]

Incubate plates at 35 ± 2°C for 18-72 hours. Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5% CO_2.

CAMP Test[10]

Non-hemoytic, bile-esculin negative streptococci or bacitracin-resistant beta-hemolytic streptococci may be tested by the CAMP test for presumptive identification as *S. agalactiae* (Lancefield group B). The inoculum may be taken from an overnight broth culture or from colonies picked from a blood agar plate. Make a single streak of *Staphylococcus aureus* ATCC 33862 across the center of a blood agar plate. If a loop is used, do not use it parallel to the agar surface, since the streak will be too wide and the results will not be satisfactory. The streptococcal isolates to be tested are inoculated by making a simple streak perpendicular to the *S. aureus* line coming as close as possible (2-3 mm), but not touching it. Several streptococcal isolates may be tested on the same plate. Perpendicular streptococcal streaks should be 5-8 mm apart. Include a known *S. agalactiae* for a positive control and *S. pyogenes* as a negative control. The procedure should be practiced with known cultures before using it to identify unknown isolates.

NOTE: Studies on the CAMP Test have shown that the reaction is most reliable early in the shelf life of some lots of the prepared plated medium. It is recommended that *S. agalactiae* ATCC 12386 be included along with patient isolates to verify satisfactory performance.

Incubate plates in an aerobic atmosphere at 35 ± 2°C for 18-24 hours. Do not incubate anaerobically or in a CO_2 incubator. False-positive results may occur with group A streptococci when incubation is in an anaerobic or CO_2-enriched atmosphere.[10,11]

Expected Results

Hemolytic streptococci may appear translucent or opaque, grayish, small (1 mm), or large matt and mucoid (2-4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.) In reporting, approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.

- Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.
- Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
- *Listeria* produce small zones of beta hemolysis. They may be distinguished by their rod shape in stains, and by motility at room temperature.

- *Haemophilus influenzae* produces nonhemolytic, small grayish, translucent colonies with a "mousy" odor on **Trypticase** Soy Agar (TSA II) with 5% Rabbit Blood.
- Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

CAMP Test

A positive CAMP reaction is indicated by an arrowhead or triangular shaped area of increased hemolysis which forms around the end of the streptococcal streak line closest to the *S. aureus* growth. The streptococcal growth must be within the wide zone of partial hemolysis that surrounds the *S. aureus* growth. A negative reaction may appear as a bullet-shaped zone of slightly increased hemolysis or as no increased hemolysis.

Bacitracin-negative, CAMP-positive, beta-hemolytic streptococci may be reported as presumptive group B streptococci. CAMP-positive group A species may be differentiated from group B streptococci by hemolysis, bacitracin susceptibility, and hippurate hydrolysis. Group B streptococci tend to produce larger colonies and have less pronounced zones of beta hemolysis than other beta-hemolytic strains, and some group B strains are nonhemolytic.[9]

References

1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Vera and Power. 1980. *In* Lennette, Balows, Hausler and Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
4. Bernheimer, Linder and Avigad. 1979. Infect. Immun. *23*:838.
5. Krumweide and Kuttner. 1938. J. Exp. Med. *67*:429.
6. Vera. 1971. Health Lab Sci. *8*:176.
7. Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.
8. Nash and Krenz. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
9. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
10. Darling. 1975. J. Clin. Microbiol. *1*:171.
11. Facklam and Washington. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Tryptic Soy Blood Agar Base No. 2

BAM COMPF

Cat. No. 227300 Dehydrated – 500 g
227200 Dehydrated – 10 kg

Difco™ Tryptic Soy Blood Agar Base EH

BAM COMPF

Cat. No. 228300 Dehydrated – 500 g
228200 Dehydrated – 10 kg

BBL™ Trypticase™ Soy Agar, Modified (TSA II)

BAM COMPF

Cat. No. 212305 Dehydrated – 500 g
297941 Prepared Pour Tubes, 20 mL – Ctn. of 100

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

BAM BS12 CCAM CMPH2 MCM9 USDA

United States and Canada
Cat. No. 221239 Prepared Plates – Pkg. of 20*
221261 Prepared Plates – Ctn. of 100*

Europe
Cat. No. 254053 Prepared Plates – Pkg. of 20*
254087 Prepared Plates – Ctn. of 120*

Japan
Cat. No. 251239 Prepared Plates – Pkg. of 20*
251261 Prepared Plates – Ctn. of 100*
251240 Prepared Plates – Ctn. of 200*

BBL™ Trypticase™ Soy Agar with 10% Sheep Blood (TSA II)

Cat. No. 221162 Prepared Plates – Pkg. of 20*
221260 Prepared Plates – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)// Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No. 221292 Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Group A Selective Strep Agar with 5% Sheep Blood (ssA™)

Cat. No. 221783 Prepared Bi-Plate Dishes – Pkg. of 20*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Chocolate II Agar

BS12 CMPH2 MCM9

United States and Canada
Cat. No. 221302 Prepared **I Plate**™ Dishes – Pkg. of 20*
221303 Prepared **I Plate**™ Dishes – Ctn. of 100*

Europe
Cat. No. 251302 Prepared **I Plate**™ Dishes – Pkg. of 20*

Japan
Cat. No. 251302 Prepared **I Plate**™ Dishes – Pkg. of 20*
251303 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Levine EMB Agar

BS12 CMPH2 MCM9

Cat. No. 221286 Prepared **I Plate**™ Dishes – Pkg. of 20*
221289 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//MacConkey II Agar

BS12 CMPH2 MCM9

United States and Canada
Cat. No. 221290 Prepared **I Plate**™ Dishes – Pkg. of 20*
221291 Prepared **I Plate**™ Dishes – Ctn. of 100*

Europe
Cat. No. 251290 Prepared **I Plate**™ Dishes – Pkg. of 20*

Japan
Cat. No. 251290 Prepared **I Plate**™ Dishes – Ctn. of 20*
251572 Prepared **I Plate**™ Dishes – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//MacConkey II Agar with MUG

Cat. No. 221949 Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Chocolate II Agar//MacConkey II Agar

Cat. No. 299580 Prepared **Y Plate**™ Dishes – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Horse Blood (TSA II)

United States and Canada
Cat. No. 221372 Prepared Plates – Pkg. of 20*

Europe
Cat. No. 212099 Prepared Plates – Pkg. of 20*

BBL™ Trypticase™ Soy Agar with 5% Rabbit Blood (TSA II)

Cat. No. 221356 Prepared Plates – Pkg. of 20*

BBL™ Trypticase™ Soy Agar (TSA II) with Defibrinated Sheep Blood Slant

Cat. No. 220830 Prepared Slants – Pkg. of 10*
220831 Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*

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Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) with Ampicillin

Intended Use

Trypticase Soy Agar with 5% Sheep Blood (TSA II) with Ampicillin is used for the isolation of *Aeromonas* spp.

Summary and Explanation

Aeromonas spp. are widely distributed in nature, primarily occurring in natural fresh and salt waters, where they infect animals, amphibians, reptiles and fish.[1] They have been recovered from a variety of specimens; gastroenteritis, however, is the most common infection associated with this organism.[2]

Aeromonas spp. grow on standard media used for the isolation of gram-negative bacilli (e.g., blood agar and MacConkey Agar), but some strains are reported to be inhibited on selective media used to isolate *Salmonella*, *Shigella* and *Campylobacter*.[2] Isolation is facilitated, however, on 5% sheep blood agar containing ampicillin as a selective agent.[1]

Principles of the Procedure

The combination of casein and soy peptones in the **Trypticase** Soy Agar base renders the medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood supplies nutrients and, simultaneously, it allows detection of hemolytic reactions. Ampicillin is active against gram-negative as well as gram-positive organisms, although most *Aeromonas* spp. are resistant to it. Agar is the solidifying agent.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 35 ± 2°C in an aerobic atmosphere for 18-48 hours.

Expected Results

Colonies of *Aeromonas* may be round, raised, with an entire edge, smooth surface and may be surrounded by a zone of beta hemolysis. Gram staining, biochemical tests and other procedures should be performed to confirm findings.

Limitations of the Procedure

1. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for identification purposes. Consult appropriate references for detailed information and recommended procedures.[1-3]
2. Ampicillin-susceptible species of *Aeromonas* have been reported.[1, 4]

References

1. Horneman, Ali and Abbott. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
3. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
4. Carnahan, Chakraborty, Fanning, Verma, Ali, Janda and Joseph. 1991. J. Clin. Microbiol. 29:1206.

Availability

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) with Ampicillin

Cat. No. 297346 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) with Gentamicin

Intended Use

Trypticase Soy Agar with 5% Sheep Blood (TSA II) with Gentamicin is used for the isolation of *Streptococcus pneumoniae* from clinical specimens.

Summary and Explanation

Streptococcus pneumoniae is the leading cause of bacterial pneumonia and a cause of meningitis, endocarditis, otitis media and sinusitis.[1]

To improve the recovery of *S. pneumoniae* from clinical specimens, gentamicin has been incorporated into a general growth medium enriched with sheep blood. Dilworth et al. and Sondag et al. reported that supplementing sheep blood agar with gentamicin increased the recovery of *S. pneumoniae* from respiratory secretions by inhibiting the growth of bacteria that masked the presence of pneumococcal colonies.[2, 3]

Trypticase Soy Agar with 5% Sheep Blood (TSA II) with Gentamicin improves the recovery of *Streptococcus pneumoniae* from clinical specimens by inhibiting the growth of bacteria that could mask its presence, including staphylococci and gram-negative bacilli. The concentration of gentamicin has been reduced to 2.5 mg/L in this medium because higher concentrations of gentamicin may inhibit the growth of some strains of *S. pneumoniae*.

Principles of the Procedure

The combination of casein and soy peptones renders the medium highly nutritious by supplying organic nitrogen, particularly

amino acids and longer-chained peptides. Sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood supplies nutrients necessary to support the growth of fastidious organisms and to detect hemolytic reactions while also inhibiting the growth of *Haemophilus haemolyticus*, a bacterium commonly found in nose and throat specimens that is indistinguishable from beta-hemolytic streptococci.[1] Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria. Agar is the solidifying agent.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 35°C in a CO_2-enriched atmosphere for 18-48 hours.

Expected Results

Staphylococci and gram-negative bacteria are inhibited. Circular, flat, translucent colonies surrounded by zones of alpha hemolysis may be presumptively identified as *Streptococcus pneumoniae*. However, when the colonies are young, they may be dome-shaped and may be confused with viridans streptococci, which will also grow on this medium. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

References

1. Spellerberg and Brandt. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed.), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Dilworth, Stewart, Gwaltney, Hendley and Sande. 1975. J. Clin. Microbiol. 2:453.
3. Sondag, Morgens, Hoppe and Marr. 1977. J. Clin. Microbiol. 5:397.

Availability

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) with Gentamicin

Cat. No. 297457 Prepared Plates – Pkg of 20*

**Store at 2-8°C.*

Bacto™ Tryptic Soy Broth/Trypticase™ Soy Broth (Soybean-Casein Digest Medium) Trypticase™ Soy Broth with 6.5% Sodium Chloride Trypticase™ Soy Broth with 5% Fildes Enrichment Bacto™ Tryptic Soy Broth without Dextrose

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Intended Use

Tryptic (**Trypticase**) Soy Broth (Soybean-Casein Digest Medium) is a general purpose medium used in qualitative procedures for the cultivation of fastidious and nonfastidious microorganisms from a variety of clinical and nonclinical specimens.

Trypticase Soy Broth with 6.5% Sodium Chloride is used to differentiate *Enterococcus* spp. from the *Streptococcus bovis* group of streptococci.

Trypticase Soy Broth with 5% Fildes Enrichment is used for the cultivation of fastidious organisms; e.g., *Haemophilus influenzae*.

Tryptic Soy Broth without Dextrose, a low carbohydrate formulation of Tryptic Soy Broth, is used for cultivating fastidious and nonfastidious microorganisms.

Tryptic (Trypticase) Soy Broth meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Tryptic (**Trypticase**) Soy Broth (TSB) is a nutritious medium that will support the growth of a wide variety of microorganisms, including common aerobic, facultative and anaerobic bacteria and fungi.[4-7] This formulation is included in the *USP* as a medium for use in performing microbial enumeration tests and tests for specified microorganisms when testing nonsterile pharmaceutical products.[1]

TSB was chosen by the USDA Animal and Plant Health Inspection Service for detecting viable bacteria in live vaccines.[8] TSB is recommended for testing bacterial contaminants in cosmetics[9,10] and complies with established standards in the food industry.[10-16]

Because of its capacity for growth promotion, TSB is also recommended for use as the inoculum broth for disc diffusion and agar dilution antimicrobial susceptibility testing as standardized by the Clinical and Laboratory Standards Institute (CLSI).[17,18]

Trypticase Soy Broth with 6.5% Sodium Chloride is used to differentiate the enterococcal species from the *S. bovis* group of streptococci by the 6.5% NaCl tolerance test.[19]

Trypticase Soy Broth supplemented with 5% Fildes Enrichment provides growth factors necessary for the cultivation of fastidious organisms.[20]

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™/Bacto™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Tryptic Soy Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in purified water upon warming. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 3.0% Solution at 25°C:	pH 7.3 ± 0.2

Difco™ Tryptic Soy Broth (prepared bottles)

Appearance:	Light to medium tan yellow, clear to trace hazy.
Reaction at 25°C:	pH 7.3 ± 0.2

Bacto™ Tryptic Soy Broth without Dextrose

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.75% solution, soluble in purified water upon warming. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 2.75% Solution at 25°C:	pH 7.3 ± 0.2

Uninoculated Tube

Staphylococcus epidermidis ATCC™ 12228

Cultural Response

Bacto™ Tryptic Soy Broth

Prepare the medium per label directions. Inoculate and incubate at 30-35°C for 18-72 hours (up to 5 days for *A. brasiliensis* and *C. albicans*). Prepare duplicate cultures of *A. brasiliensis*, *B. subtilis* and *C. albicans* and incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	10-100	Fair to good
Staphylococcus epidermidis	12228	10-100	Good
Streptococcus pneumoniae	6305	10-100	Good
Streptococcus pyogenes	19615	10-100	Good
Aspergillus brasiliensis (niger)	16404	<100	Growth (30-35°C)
Aspergillus brasiliensis (niger)	16404	<100	Growth (20-25°C)
Bacillus subtillis	6633	<100	Growth (30-35°C)
Bacillus subtillis	6633	<100	Growth (20-25°C)
Candida albicans	10231	<100	Growth (30-35°C)
Candida albicans	10231	<100	Growth (20-25°C)
Escherichia coli	8739	<100	Growth
Pseudomonas aeruginosa	9027	<100	Growth
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	<100	Growth
Staphylococcus aureus	6538	<100	Growth

Difco™ Tryptic Soy Broth (prepared bottles)

Inoculate and incubate at 30-35°C for 18-24 hours (up to 3 days for *B. subtilis*). For (*) cultures incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
*Aspergillus brasiliensis (niger)**	16404	10-100	Growth (20-25°C)
Bacillus subtillis	6633	10-100	Growth (30-35°C)
*Bacillus subtillis**	6633	10-100	Growth (20-25°C)
*Candida albicans**	10231	10-100	Growth (20-25°C)
Escherichia coli	8739	10-100	Growth
Pseudomonas aeruginosa	9027	10-100	Growth
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10-100	Growth
Staphylococcus aureus	6538	10-100	Growth

Bacto™ Tryptic Soy Broth without Dextrose

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	30-300	Fair to good
Staphylococcus epidermidis	12228	30-300	Good
Streptococcus pneumoniae	6305	30-300	Good
Streptococcus pyogenes	19615	30-300	Good

Continued

Tryptic Soy Broth without Dextrose, a modification of TSB, is a basal medium to which carbohydrates may be added for use in fermentation studies. Phenol red and other indicators may also be added.

Principles of the Procedure

Enzymatic digests of casein and soybean provide amino acids and other complex nitrogenous substances. Dextrose is an energy source. Sodium chloride maintains the osmotic equilibrium. Dibasic potassium phosphate acts as a buffer to control pH.

Identity Specifications

BBL™ Trypticase™ Soy Broth

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 3.0% solution, soluble in purified water upon warming. Solution is light, tan to yellow, clear to slightly hazy.

Prepared Appearance: Light, tan to yellow, clear to slightly hazy.

Reaction of 3.0% Solution at 25°C: pH 7.3 ± 0.2

BBL™ Trypticase™ Soy Broth (prepared bottles)

Appearance: Light to medium tan yellow, clear to trace hazy.

Reaction at 25°C: pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Broth

Prepare the medium per label directions. Inoculate tubes and incubate at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*). Prepare duplicate cultures of *A. brasiliensis, B. subtilis* and *C. albicans* and incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	<100	Growth (30-35°C)
Aspergillus brasiliensis (niger)	16404	<100	Growth (20-25°C)
Bacillus subtilis	6633	<100	Growth (30-35°C)
Bacillus subtilis	6633	<100	Growth (20-25°C)
Candida albicans	10231	<100	Growth (30-35°C)
Candida albicans	10231	<100	Growth (20-25°C)
Escherichia coli	8739	<100	Growth
Pseudomonas aeruginosa	9027	<100	Growth
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	<100	Growth
Staphylococcus aureus	6538	<100	Growth

BBL™ Trypticase™ Soy Broth (prepared bottles)

Inoculate and incubate at 35-37°C for 48 hours. Incubate (*) cultures at 30-35°C for up to 3 days. Incubate (**) cultures at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	<100	Growth
Staphylococcus aureus	25923	<100	Growth
*Aspergillus brasiliensis (niger)***	16404	<100	Growth (20-25°C)
*Bacillus subtillis**	6633	<100	Growth (30-35°C)
*Bacillus subtillis***	6633	<100	Growth (20-25°C)
*Candida albicans***	10231	<100	Growth (20-25°C)
*Pseudomonas aeruginosa**	9027	<100	Growth (30-35°C)
*Staphylococcus aureus**	6538	<100	Growth (30-35°C)

The addition of 6.5% sodium chloride to **Trypticase** Soy Broth permits the differentiation of salt-tolerant enterococci, which are resistant to the high salt content, from the salt-intolerant *S. bovis* group and other streptococcal species. At this concentration, the sodium chloride is a selective agent that interferes with membrane permeability and osmotic and electrokinetic equilibria.[4]

Fildes Enrichment is a peptic digest of sheep blood that supplies the X (hemin) and V (nicotinamide adenine dinucleotide, NAD) factors necessary for the growth of *H. influenzae*.

Dextrose is omitted from the formula for Tryptic Soy Broth without Dextrose to permit use of the medium in fermentation studies. The carbohydrate concentration used most frequently in fermentation reactions is 0.5% or 1%.

Tryptic Soy Broth and **Trypticase** Soy Broth are provided as prepared media in a variety of bottle styles. In addition, Tryptic Soy Broth is provided as a Sterile Pack Bottle; i.e., the bottle has been terminally sterilized inside of autoclavable double-bags. All varieties of bottled TSB conform with requirements for Ready-To-Use Media as described in the *USP*.

Formulae

Bacto™ Tryptic Soy Broth (Soybean-Casein Digest Medium)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Papaic Digest of Soybean	3.0	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g

BBL™ Trypticase™ Soy Broth (Soybean-Casein Digest Broth)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Papaic Digest of Soybean	3.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Dextrose	2.5	g

Bacto™ Tryptic Soy Broth without Dextrose

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Enzymatic Digest of Soybean Meal	3.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Bacto™ Tryptic Soy Broth – 30 g;
   BBL™ Trypticase™ Soy Broth – 30 g;
   Bacto™ Tryptic Soy Broth without Dextrose – 27.5 g.
   Mix thoroughly.
2. Warm gently until solution is complete.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[5,7,17-19]

For food, dairy or cosmetic samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[9-16]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using Tryptic/**Trypticase** Soy Broth.[17-19]

For food, dairy or cosmetic samples, refer to appropriate standard references for details on test methods using Tryptic/**Trypticase** Soy Broth.[9-16]

For pharmaceutical samples, refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products and performing microbial enumeration tests and tests for specific organisms using Tryptic/**Trypticase** Soy Broth.[1]

Swab specimens may be inserted into the medium after inoculation of appropriate plated media. For liquid specimens, use a sterile inoculating loop to transfer a loopful of the specimen to the broth medium. Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.

Incubate the tubes and bottles with loosened caps at 35 ± 2°C aerobically with or without supplementation with carbon dioxide. Tubed and bottled media intended for the cultivation of anaerobes should be incubated under anaerobic conditions. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak™** EZ anaerobic systems or equivalent alternative system. Examine for growth after 18-24 hours and 42-48 hours of incubation.

Expected Results

Growth in broth media is indicated by the presence of turbidity compared to an uninoculated control. Broth cultures should be held for at least a week before discarding as negative.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
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11. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
12. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
13. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
14. Health Canada. The compendium of analytical methods, online. Food Directorate, Health Products and food Branch, Health Canada, Ottawa, Ontario Canada.
15. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.
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17. Clinical and Laboratory Standards Institute. 2006. Approved Standard M2-A9: Performance standards for antimicrobial disk susceptibility tests, 9th ed., CLSI, Wayne, Pa.
18. Clinical and Laboratory Standards Institute. 2006. Approved Standard M7-A7: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed., CLSI, Wayne, Pa.
19. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
20. Fildes. 1920. Br. J. Exp. Pathol. *1*:129.

Availability

Bacto™ Tryptic Soy Broth (Soybean-Casein Digest Medium)

AOAC BAM BS12 CCAM CLSI CMPH2 COMPF EP EPA ISO JP MCM9 SMD USDA USP

Cat. No.	211824	Dehydrated – 100 g†
	211825	Dehydrated – 500 g†
	211822	Dehydrated – 2 kg†
	211823	Dehydrated – 10 kg†
	290612	Prepared Bottles (wide mouth), 90 mL – Pkg. of 10†
	290613	Prepared Bottles (wide mouth), 100 mL – Pkg. of 10†
	257213	Sterile Pack Bottles (double bagged), 100 mL – Pkg. of 10

Europe

Cat. No.	257423	Prepared Tubes, 13 mL – Pkg. of 25†
	254960	Prepared Bottles (double-strength), 50 mL – Pkg. of 25
	257248	Prepared Bottles, 100 mL – Pkg. of 10†
	257265	Prepared Bottles (double bagged), 100 mL – Pkg. of 10†
	257276	Prepared Bottles, 100 mL (screw cap) – Pkg. of 25†
	257247	Prepared Bottles, 100 mL (tear off seal with stopper) – Pkg. of 25†
	257307	Prepared Bottles (ETO), 100 mL – Pkg. of 44†
	257316	Prepared Bottles (wide mouth), 150 mL – Pkg. of 25†
	257412	Prepared Bottles, 300 mL – Pkg. of 10†
	257413	Prepared Bottles, 500 mL – Pkg. of 4†
	257414	Prepared Bottles, 600 mL – Pkg. of 4†
	257291	Prepared Bottles (double bagged), 800 mL – Pkg. of 4†

BBL™ Trypticase™ Soy Broth (Soybean-Casein Digest Broth)

AOAC BAM BS12 CCAM CLSI CMPH2 COMPF EP EPA ISO JP MCM9 SMD USDA USP

Cat. No.	211768	Dehydrated – 500 g†
	296264	Sterile, Dehydrated – 500 g
	211771	Dehydrated – 5 lb (2.3 kg)†
	211772	Dehydrated – 25 lb (11.3 kg)†
	295634	Prepared Tubes, 1 mL (K Tubes) – Ctn. of 100
	221815	Prepared Tubes, 2 mL (K Tubes) – Ctn. of 100
	221715	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10
	221716	Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100
	221092	Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10
	221093	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100
	299936	Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100†
	221823	Prepared Tubes, 15 mL (A Tubes) – Ctn. of 100
	299749	Prepared Tubes, 20 mL (A Tubes) – Ctn. of 100†
	297811	Prepared Tubes, 21 mL (A Tubes) – Pkg. of 10
	297380	Prepared Bottles, 30 mL – Each
	299107	Prepared Bottles, 100 mL (serum bottle) – Pkg. of 10†
	299416	Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10†
	257411	Prepared Bottles, 200 mL (flip cap with stopper) – Pkg. of 10
	299113	Prepared Bottles, 500 mL – Pkg. of 10†

Mexico

Cat. No.	252605	Prepared Tubes, 10 mL
	252736	Prepared Tubes, 5 mL

BBL™ Trypticase™ Soy Broth with 6.5% Sodium Chloride

Cat. No.	211351	Prepared Tubes (K Tubes) – Ctn. of 100

BBL™ Trypticase™ Soy Broth with Fildes Enrichment

Cat. No.	221403	Prepared Tubes (K Tubes) – Pkg. of 10*
	221404	Prepared Tubes (K Tubes) – Ctn. of 100*

Bacto™ Tryptic Soy Broth without Dextrose

BAM

Cat. No.	286220	Dehydrated – 500 g
	286210	Dehydrated – 10 kg

**Store at 2-8°C.*
†QC testing performed according to USP/EP/JP performance specifications.

Trypticase™ Soy Broth with 0.15% Agar

Intended Use

Trypticase Soy Broth (TSB) with 0.15% Agar is a general-purpose medium for cultivation of fastidious and nonfastidious microorganisms, especially anaerobic bacteria.

Summary and Explanation

TSB is a nutritious medium that supports the growth of a wide variety of microorganisms, including common aerobic, anaerobic and facultative bacteria and fungi.[1-3] The addition of agar enhances the cultivation of some microorganisms, particularly anaerobes from root canal and other clinical specimens.[1]

Principles of the Procedure

TSB contains enzymatic digests of casein and soybean meal to provide nitrogenous substances. Dextrose is a source of energy, and sodium chloride provides osmotic equilibrium.

Supplementing TSB with agar is useful for the cultivation of anaerobes. The agar in the medium retards the absorption of oxygen by reducing convection currents in the medium.

Procedure

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak**™ EZ anaerobic systems or an alternative anaerobic system.[4]

The organisms to be cultivated must first be isolated in pure culture on an appropriate plated or slanted medium.

Using a sterile inoculating loop or needle, transfer fresh growth from the plate or slant to the tubed medium to achieve the desired concentration of viable organisms. Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.

Tubed media intended for isolation and cultivation of anaerobes should be incubated under anaerobic conditions for up to 7 days.

Expected Results

Growth in tubes is indicated by the presence of turbidity compared to an uninoculated control.

If growth appears, cultures should be examined by Gram staining and subculturing onto appropriate media; e.g., a **Trypticase**™ Soy Agar with 5% Sheep Blood (TSA II) plate and/or Chocolate II Agar plate, EMB Agar or MacConkey II Agar plate, etc. If anaerobes are suspected, subcultures should be incubated anaerobically, as in a **GasPak** EZ anaerobic system.

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Fredette, Auger and Forget. 1961. Can . Med. Assoc. J. *84*:164.
3. Forbes, Sahm, and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
4. Seip and Evans. 1980. J. Clin. Microbiol. *11*:226.

Availability

BBL™ Trypticase™ Soy Broth with 0.15% Agar

Cat. No.	298263	Prepared Tubes (K Tubes), 9 mL – Ctn. of 100

Trypticase™ Soy Broth with 20% Glycerol

Intended Use
This medium is used in the long-term frozen maintenance of bacterial stock cultures.

Summary and Explanation
Trypticase Soy Broth, a nutritious medium supplemented with glycerol, may be used as a maintenance medium for the preservation of bacterial cultures.[1, 2]

Principles of the Procedure
Enzymatic digests of protein substrates act as protective colloids. Glycerol is a cryoprotective agent that provides intracellular and extracellular protection against freezing.[2]

Procedure
Using a sterile swab or inoculating loop, remove fresh growth from the plated or slanted medium and suspend in the broth maintenance medium to achieve the desired concentration of viable cells. Freeze suspension immediately at -20°C or below. Consult texts for detailed information about preparing stock cultures of specific organisms.[2-4]

Expected Results
Bacterial stock cultures frozen and stored at -20°C or below will remain viable for several months, and some may remain viable for years.

References
1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Gherna. 1994. *In* Gerhardt, Murray, Wood and Krieg (ed.). Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
3. Norris and Ribbons (ed.). 1970. Methods for microbiology, vol. 3A. Academic Press, Inc., New York, N.Y.
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Availability
BBL™ Trypticase™ Soy Broth with 20% Glycerol

Cat. No.	296346	Prepared Tubes (K Tubes), 1.5 mL – Pkg. of 10
	297808	Prepared Tubes (K Tubes), 1.5 mL – Ctn. of 100
	297352	Prepared Tubes (C Tubes), 10 mL – Ctn. of 100

Tryptone • Trypticase™ Peptone

(See Casitone)

Tryptone Glucose Extract Agar • m TGE Broth

Intended Use
Tryptone Glucose Extract Agar is used for cultivating and enumerating microorganisms in water and dairy products.

m TGE Broth, also known as membrane Tryptone Glucose Extract Broth, is used for enumerating microorganisms by membrane filtration.

Summary and Explanation
In the 1930s, Bower and Hucker[1] developed a medium for detecting bacteria in milk and other dairy products. Many investigators compared the performance of Tryptone Glucose Skim Milk Agar to Nutrient Agar for estimating bacteria in milk and other dairy products.[2-4] Prickett[5] used a glucose agar containing tryptone to study thermophilic bacteria in milk. This medium, described in *Standard Methods of Milk Analysis*,[6] was prepared in the dehydrated form as Yeast Dextrose Agar. The American Public Health Association (APHA) adopted Tryptone Glucose Extract Agar for use in testing milk and dairy products in 1948.[7] For many years, Tryptone Glucose Extract Agar with added milk remained the standard methods medium for dairy products[8] and was also adopted for testing water.[9] It is currently recommended in the *Compendium of Methods for the Microbiological Examination of Foods* for performing the heterotrophic plate count procedure in testing bottled water.[10]

m TGE Broth is a nonselective nutrient medium for the determination of bacterial counts by the membrane filter method. The broth has the same formulation as the agar except that the broth contains no agar and the ingredients are at twice the concentration.

Principles of the Procedure
Nutrients, including amino acids, carbon compounds, carbohydrates, minerals and trace substances, are supplied by the tryptone, beef extract and dextrose. Agar is the solidifying agent in Tryptone Glucose Extract Agar.

Formulae
Difco™ Tryptone Glucose Extract Agar

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Tryptone	5.0	g
Dextrose (Glucose)	1.0	g
Agar	15.0	g

Difco™ m TGE Broth

Approximate Formula* Per Liter		
Beef Extract	6.0	g
Tryptone	10.0	g
Dextrose (Glucose)	2.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Tryptone Glucose Extract Agar

Dehydrated Appearance:	Light to medium tan, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in purified water upon boiling. Solution is light amber, clear to slightly opalescent.
Prepared Appearance:	Light amber, clear to slightly opalescent.
Reaction of 2.4% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ m TGE Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	1.8% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Medium amber, clear to very slightly opalescent.
Reaction of 1.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

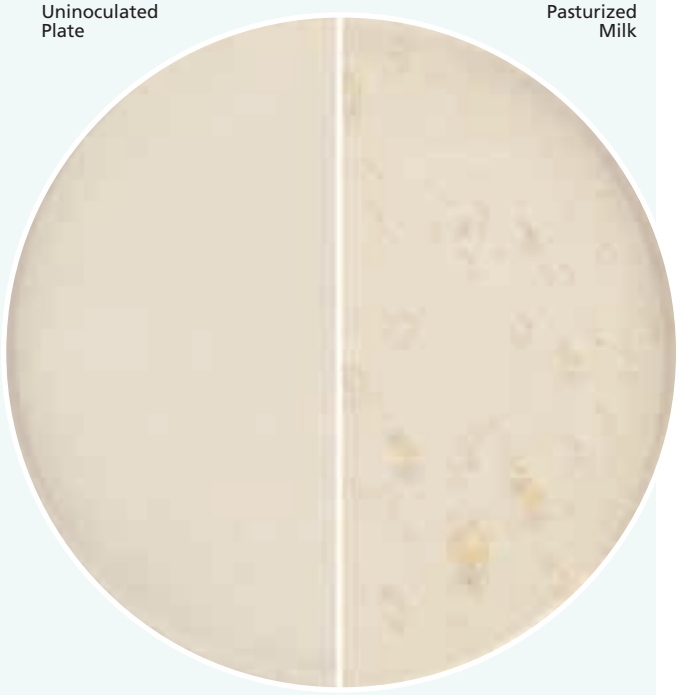
Difco™ Tryptone Glucose Extract Agar

Prepare the medium per label directions in parallel with a control (approved) lot of medium. Inoculate with serial dilutions of pasteurized and raw milk samples using the pour plate technique and incubate at 32 ± 1°C for 47-49 hours. Recovery of bacteria from the milk samples should be comparable for both the test and control lots.

Difco™ m TGE Broth

Prepare the medium per label directions. Inoculate using the membrane filter technique and incubate at 35 ± 2°C for 18-24 hours in a humid atmosphere.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	30-300	Good
Staphylococcus aureus	25923	30-300	Good



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Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ Tryptone Glucose Extract Agar – 24 g;
   Difco™ m TGE Broth – 18 g.
   Mix thoroughly.
2. For agar, heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult the reference for information regarding the processing and inoculation of bottled water samples.[10]

Agar (Pour Plate)

Usually 1 mL samples of appropriate dilutions of the test sample are pipetted into sterile Petri dishes and molten, cooled Tryptone Glucose Extract Agar is added followed by gentle mixing to distribute the sample dilution throughout the agar. Incubate hardened plates at 32 ± 1°C for 47-49 hours, or as specified in standard methods (35 ± 0.5°C for at least 72 hours).[10]

Broth (Membrane Filtration)

Inoculate medium using the membrane filter technique and incubate at 35 ± 2°C for 18-24 hours in a humid atmosphere.

Expected Results

Follow recommended procedures for counting colonies and reporting results.[10]

Because bacteria found in bottled water demonstrate a prolonged lag phase during adaptation to growth on Tryptone Glucose Extract Agar, extended incubation beyond 48 hours may be required.[10]

References

1. Bowers and Hucker. 1935. Tech. Bull. 228. New York State Agr. Exp. Sta., Geneva, N.Y.
2. Yale. 1938. Am. J. Pub. Health *28*:148.
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Availability

Difco™ Tryptone Glucose Extract Agar

AOAC COMPF

Cat. No. 223000 Dehydrated – 500 g

Japan

Cat. No. 252127 Prepared Plates – Pkg. of 20*

Mexico

Cat. No. 252740 Prepared Plates (60 × 15 mm-style) – Pkg. of 20*

Difco™ m TGE Broth

Cat. No. 275010 Dehydrated – 100 g
275020 Dehydrated – 500 g

**Store at 2-8° C.*

Tryptone Water

Intended Use

Tryptone Water is recommended for use in the detection of *Escherichia coli* in food and water samples based on indole production.

Summary and Explanation

Tryptone Water is based on the Tryptone Water formula described in ISO standard 7251.[1] In this procedure, Tryptone Water is used with Lauryl Tryptose (or Sulfate) Broth and EC Broth to determine the most probable number (MPN) of *E. coli* present in the sample. Gas production in both media and indole production in Tryptone Water is used as the basis for this presumptive *E. coli* test.

Tryptone Water may also be used for differentiation of other bacteria based on indole production.

Principles of the Procedure

Tryptone Water contains both tryptone (1%) and sodium chloride. Due to its high tryptophan content, tryptone is suitable for use in detecting indole production by bacteria. Tryptophan is hydrolyzed and deaminated to produce indole, pyruvic acid and ammonia.[2] Indole can then be detected by the addition of either Kovacs' or Ehrlich's Reagent, which contain an aldehyde group. The aldehyde group combines with indole to produce a red color in the alcohol layer. Sodium chloride is added to the medium to provide a suitable osmotic environment.

User Quality Control

Identity Specifications

Difco™ Tryptone Water

Dehydrated Appearance:	White to light beige, free flowing, homogeneous.
Solution:	1.5% solution, soluble in purified water. Solution is pale to medium amber, clear to slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to slightly opalescent.
Reaction of 1.5% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Tryptone Water

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. Add 0.5 mL Indole Reagent (Kovacs) to the tubes to test for indole production. Formation of a red color denotes a positive indole test.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	INDOLE PRODUCTION
Enterobacter cloacae	13047	10^2-3×10^2	Good	–
Escherichia coli	25922	10^2-3×10^2	Good	+

Formula

Difco™ Tryptone Water

Approximate Formula* Per Liter

Tryptone	10.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 15 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Test For Enumeration of Presumptive *E. coli*

1. Suspend one part sample in 9 parts diluent. Homogenize sample.
2. Prepare serial 10-fold dilutions to 10^{-6} using 1 mL of homogenate and 9 mL of diluent. Mix each dilution thoroughly.
3. Transfer 10 mL of test sample or initial suspension to each of 3 tubes of double-strength Lauryl Tryptose Broth (LTB). Repeat using 3 tubes of single-strength LTB. Mix well.
4. For each of the serial 10-fold dilutions, transfer 10 mL of test sample to each of 3 tubes of double-strength LTB. Repeat using 3 tubes of single-strength LTB. Mix well.
5. Incubate all tubes of LTB at 35-37°C for 24 ± 2 hours and up to 48 hours, if necessary, observing tubes for gas formation.
6. Inoculate one 3-mm loopful of broth from each tube in Step 5 showing gas formation to 10 mL of EC Broth warmed to 45°C.
7. Incubate the EC Broth tubes in a water bath at 45°C for 24 ± 2 hours (up to 48 hours if necessary), observing for gas formation.
8. Inoculate one 3-mm loopful of broth from each tube in Step 7 showing gas formation to 5-10 mL of Tryptone Water warmed to 45°C.

9. Incubate Tryptone Water tubes in a water bath at 45°C for 48 hours.
10. Add 0.5 mL of Indole Reagent to Tryptone Water tubes, mix well and examine after 1 minute.

Indole Determination Using Pure Cultures

1. Inoculate Tryptone Water using a light inoculum of an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C with loosened caps for 18-24 hours.
3. Add 0.5 mL of Indole Reagent (Kovacs) directly to the tube and agitate. Allow tubes to stand for 5-10 minutes.

Expected Results

Test For Enumeration of Presumptive *E. coli*

For each dilution, record tubes as positive if a red ring forms at the top of the medium indicating indole production. Determine the MPN (Most Probable Number) of *E. coli* present in the sample based on the number of tubes that are positive for both gas and indole. Consult the appropriate 3-tube MPN table.[1]

Indole Determination Using Pure Cultures

Examine tubes for the formation of a red ring at the top of the tube indicating indole production.

Limitations of the Procedure

1. Detection of *E. coli* in meats using Tryptone Water is a presumptive test. If confirmatory testing is required, please consult appropriate references.
2. Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production. For complete identification of the organism, further biochemical evaluation is necessary.

References

1. International Organization for Standardization. 1993. Microbiology – general guidance for enumeration of presumptive *E. coli* – most probable number technique. ISO 7251, 1993-12-15, 2nd ed. ISO, Geneva, Switzerland.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Tryptone Water

ISO

Cat. No. 264410 Dehydrated – 500 g

Difco™/BBL™ Indole Reagent

Cat. No. 261185 Droppers – 50 × 0.5 mL

Bacto™ Tryptose

Intended Use

Bacto Tryptose is an enzymatic digest of protein used in preparing microbiological culture media.

Summary and Explanation

Tryptose was originally developed as a peptone particularly adapted to growth requirements of *Brucella*. Tryptose is very useful for cultivation of streptococci, pneumococci, meningococci and other fastidious organisms, and was found to be superior to meat infusion peptone media previously used for these organisms.[1,2] Mobley et al.[3] reported that Tryptose Broth was the preferred medium for strains of *Bordetella bronchiseptica* in studies of phosphatase activity.

Tryptose has been reported as beneficial for cell culture applications. Litwin[4] found Tryptose to be suitable for supplementing a serum-free medium to grow human diploid fibroblasts. Vaughn and Fan[5] established that Tryptose provided free amino acids necessary for growth of *Spodoptera frugiperda* and *Lymantria dispar* insect cell lines. Tryptose is often used as a biomass enhancer for recombinant *E. coli* production.

Tryptose is the major ingredient and only peptone in the formulation for Tryptose Phosphate Broth (TPB), an often-used medium for various culture applications. Hata and Kojima[6] have shown TPB to be a useful supplement in culturing the nematode, *Angiostrongylus cantonensis*. TPB was also reported as a supplement to a medium for cultivating a protozoan parasite, which parasitizes vectors of Chagas' disease, on its insect cell host.[7] *Spodoptera frugiperda*, a cotton pest in Argentina[8] and several tick cell lines have also been grown using a TPB-supplemented medium.[9] Tryptose Phosphate Broth has been reported as a suitable supplement for growth of baby hamster kidney cells[10] and porcine kidney cells.[11]

Media formulations containing **Bacto** Tryptose are specified in standard methods for various applications.[12-17]

Principles of the Procedure

Bacto Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process of **Bacto** Tryptose results in assorted peptides of higher molecular weight suitable for long chain amino acid requirements. **Bacto** Tryptose provides nitrogen, amino acids and vitamins in microbiological culture media.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** Tryptose in the formula of the medium being prepared. Add product as required.

User Quality Control

Identity Specifications

Bacto™ Tryptose

Dehydrated Appearance:	Tan, free-flowing, granules.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is light amber, clear. 2.0% solution is medium amber, clear to slightly opalescent. 10.0% solution is medium to dark amber, very slightly opalescent to opalescent, may have a precipitate.
Reaction of 1.0% Solution at 25°C:	pH 7.1-7.5

Cultural Response

Biochemical Reactions

Bacto™ Tryptose

Prepare a sterile solution of **Bacto** Tryptose as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922	~10^7	Negative
Indole Production	0.1%	*Escherichia coli*	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	*Enterobacter aerogenes*	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	*Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	0.1 mL, undiluted	Positive

Growth Response

Bacto™ Tryptose

Prepare a sterile solution with 2% **Bacto** Tryptose, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Staphylococcus aureus	25923	30-300	Good
Streptococcus pneumoniae	6303	30-300	Good
Streptococcus pyogenes	19615	30-300	Good

Procedure

See appropriate references for specific procedures using **Bacto** Tryptose.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Casman. 1942. J. Bacteriol. *43*:33.
2. Casman. 1947. Am. J. Clin. Pathol. *17*:281.
3. Mobley, Chengappa, Kadel and Stuart. 1984. Can. J. Comp. Med. *48*:175.
4. Litwin. 1985. Dev. Biol. Stand. *60*:25.
5. Vaughn and Fan. 1997. In Vitro Cell. Dev. Biol. Anim. *33*:479.
6. Hata and Kojima. 1990. Exp. Parasitol. *70*:467.
7. Reduth, Schaub, and Pudney. 1989. Parasitology *98*:387.
8. Deutschmann and Jager. 1994. Enzyme Microb. Technol. *16*:506.
9. Munderloh and Kurtti. 1989. Exp. Appl. Acarol. *7*:219.
10. Prodafikas and Plavsic. 2000. Focus *22*:35.
11. Sakoda and Fukusho. 1998. In Vitro Cell Dev. Biol. Anim. *34*:53.
12. Horowitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed, online. AOAC International, Gaithersburg, Md.
13. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
14. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
15. U.S. Environmental Protection Agency (USEPA). 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, USEPA, Washington, D.C.
16. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
17. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food and Safety Inspection Service, USDA, Washington, D.C.

Availability

Bacto™ Tryptose

AOAC BAM COMPF EPA SMWW USDA

Cat. No.	211713	Dehydrated – 500 g
	211709	Dehydrated – 10 kg

Tryptose Agar • Tryptose Broth

Intended Use

Tryptose Agar is used for cultivating a wide variety of fastidious microorganisms, particularly for isolating *Brucella* according to Huddleson and Castañeda.

Tryptose Broth is used for cultivating *Brucella* and other fastidious microorganisms.

Summary and Explanation

Tryptose media, prepared without extract or infusion of meat, are recommended for the cultivation and isolation of pathogenic and saprophytic bacteria. Historically, it was considered necessary to include meat extract or infusion as a nutritional supplement in culture media. Tryptose was developed while

User Quality Control

Identity Specifications

Difco™ Tryptose Agar

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Tryptose Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.6% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 2.6% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Tryptose Agar or Tryptose Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella abortus	11192*	10^2-10^3	Good
Brucella melitensis	4309*	10^2-10^3	Good
Brucella suis	9843*	10^2-10^3	Good

**Minimally one strain of* Brucella *should be used for performance testing. These ATCC strains should be used if available.*

studying the growth requirements of *Brucella*. Huddleson[1] found tryptose media to be equal or superior to meat infusion media, providing uniformity for the cultivation and differentiation of fastidious organisms.

Tryptose media are particularly well suited for the isolation of *Brucella* from blood. Castañeda[2] studied the isolation of *Brucella* species using a broth containing 2% tryptose and 2% sodium citrate. Sodium citrate serves as an anticoagulant and assists in inactivating complement in the blood specimen.

Tryptose Broth can be used as a complete basal medium or supplemented with enrichments. Huddleson[3] used a broth containing 2% tryptose as an enrichment medium in the isolation of *Brucella* from clinical specimens. McCullough et al. reported that addition of thiamine, dextrose and iron salts increased growth of *Brucella suis*.[4] Addition of 0.1% agar to Tryptose Broth can increase growth of aerobes and anaerobes in liquid media. Blood agar may be prepared by adding 5% sterile, defibrinated sheep, horse or rabbit blood to the sterile medium.

The high productivity of tryptose media in the isolation and cultivation of *Brucella* supports use of these formulas as general-purpose media, especially when avoidance of animal tissue products is desired. Tryptose Agar with 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae.[5] For isolation of *Brucella* stains from contaminated milk, crystal violet (gentian violet) can be added to Tryptose Agar to suppress gram-positive organisms.[6] Tryptose media can be supplemented with thiamine or citrate for the cultivation and maintenance of fastidious aerobic and facultative microorganisms.[7]

Tryptose Agar is specified in the *Compendium of Methods for the Microbiological Examination of Foods*.[8] Tryptose media are recommended in the FDA *Bacteriological Analytical Manual* for serological testing.[9]

Principles of the Procedure

Tryptose peptone is a source of nitrogen and carbon. Dextrose is a source of carbohydrate. Sodium chloride maintains osmotic balance. Agar is the solidifying agent in Tryptose Agar.

Formulae

Difco™ Tryptose Agar

Approximate Formula* Per Liter

Tryptose	20.0	g
Dextrose	1.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

Difco™ Tryptose Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions[10]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

Difco™ Tryptose Agar

1. Suspend 41 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: To prepare blood agar, aseptically add 5% sterile defibrinated sheep, horse or rabbit blood. Dispense into sterile Petri dishes.

Difco™ Tryptose Broth

1. Dissolve 26 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Methodologies for the multiple applications using tryptose media are outlined in the references.

Expected Results
Refer to appropriate references and procedures for results.

Limitations of the Procedure
1. Tryptose media are general-purpose, non-selective media. Although certain diagnostic tests may be performed directly on the medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification.
2. When preparing blood agar, hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood.
3. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[11] For optimal performance, incubate tryptose media supplemented with blood under increased CO_2 or anaerobic conditions.
4. Dextrose has been shown to inhibit hemolysin production by some organisms.

References
1. Huddleson. 1943. Brucellosis in man and animals, rev. ed. The Commonwealth Fund, New York, N.Y.
2. Castañeda. 1947. Proc. Soc. Exp. Biol. Med. *64*:114.
3. Huddleson. 1939. Brucellosis in man and animals. Oxford University Press, Oxford, England.
4. McCullough, Mills, Herbst, Roessler and Brewer. 1947. J. Bacteriol. *53*:5.
5. Moyer and Holcomb. 1995. *In* Murray, Baron, Pfaller, Tenover, and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
7. Atlas. 1995. Handbook of microbiology media for the examination of food. CRC Press, Boca Raton, Fla.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, Washington, D.C.
9. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
10. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
11. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability
Difco™ Tryptose Agar
BAM CCAM COMPF

Cat. No.	264300	Dehydrated – 500 g
	264100	Dehydrated – 2 kg

Difco™ Tryptose Broth
BAM CCAM COMPF

Cat. No.	262200	Dehydrated – 500 g
	262100	Dehydrated – 10 kg

Tryptose Blood Agar Base

Intended Use
Tryptose Blood Agar Base is used with blood in isolating, cultivating and determining the hemolytic reactions of fastidious microorganisms.

Summary and Explanation
Investigations of the nutritive properties of tryptose demonstrated that culture media prepared with this peptone were superior to the meat infusion peptone media previously used for the cultivation of *Brucella*, streptococci, pneumococci, meningococci and other fastidious bacteria. Casman[1,2] reported that a medium consisting of 2% tryptose, 0.3% beef extract, 0.5% NaCl, 1.5% agar and 0.03% dextrose equaled fresh beef infusion base with respect to growth of organisms. The small amount of carbohydrate was noted to interfere with hemolytic reactions, unless the medium was incubated in an atmosphere of carbon dioxide.

Tryptose Blood Agar Base is a nutritious infusion-free basal medium typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, this base can be used as a general-purpose medium. Tryptose Blood Agar Base is included in the FDA *Bacteriological Analytical Manual* (pH adjusted to 6.8 ± 0.2).[3]

Principles of the Procedure
Tryptose is the source of nitrogen, carbon and amino acids in Tryptose Blood Agar Base. Beef extract provides additional nitrogen. Sodium chloride maintains osmotic balance. Agar is the solidifying agent.

Supplementation with 5-10% blood provides additional growth factors for fastidious microorganisms and is used to determine hemolytic patterns of bacteria.

Formula
Difco™ Tryptose Blood Agar Base
Approximate Formula* Per Liter

Tryptose	10.0	g
Beef Extract	3.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 33 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium cooled to 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable typical control cultures.

User Quality Control

Identity Specifications

Difco™ Tryptose Blood Agar Base

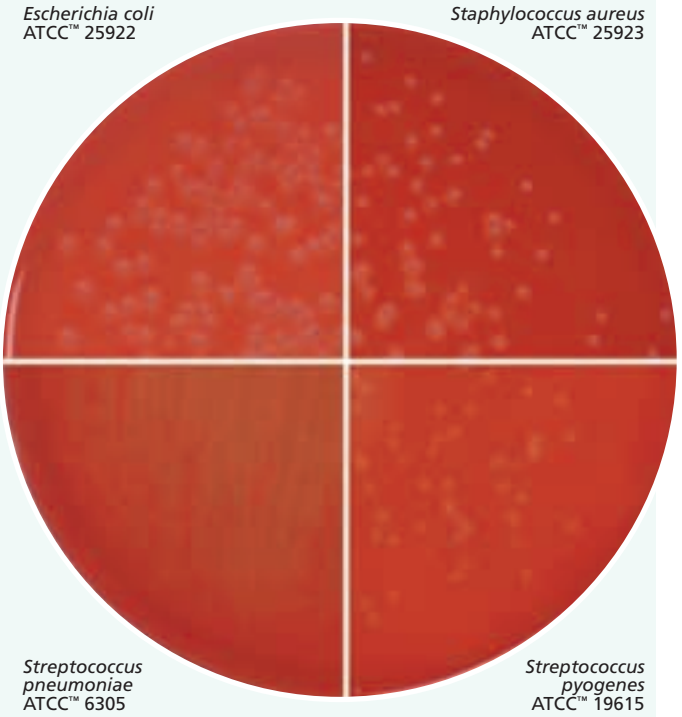
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Plain – Light amber, slightly opalescent. With 5% sheep blood – Cherry red, opaque.
Reaction of 3.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Tryptose Blood Agar Base

Prepare the medium per label directions without (plain) and with 5% sterile defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C for 18-48 hours (blood plates under 5-10% CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB	HEMOLYSIS 18-48 HR
Escherichia coli	25922	10^2-10^3	Good	Good	Beta
Neisseria meningitidis	13090	10^2-10^3	None to poor	Good	None
Staphylococcus aureus	25923	10^2-10^3	Good	Good	Beta
Streptococcus pneumoniae	6305	10^2-10^3	Fair to good	Good	Alpha
Streptococcus pyogenes	19615	10^2-10^3	Fair to good	Good	Beta



Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions of both oxygen-stable and oxygen-labile streptolysins.[4]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.
3. Examine plates for growth and hemolytic reactions after 18-24 and 48-hour incubation.

Expected Results

Four different types of hemolysis on blood agar media can be described:[5]

a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish discolorization of the medium.
b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.
d. Alpha-prime (ὰ)-hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Blood Agar Base Media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
2. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit blood agar and alpha-hemolytic on sheep blood agar.[4]
3. Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar, and must be distinguished from colonies of beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[6]
4. The atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[3] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.
5. Hemolytic patterns may vary with the source of animal blood or type of base medium used.[4]

References

1. Casman. 1942. J. Bacteriol. *43*:33.
2. Casman. 1947. Am. J. Clin. Pathol. *17*:281.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
5. Isenberg. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.

Availability

Difco™ Tryptose Blood Agar Base

BAM

Cat. No.	223220	Dehydrated – 500 g
	223210	Dehydrated – 2 kg

Bacto™ Tryptose Phosphate Broth

Intended Use

Bacto Tryptose Phosphate Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

Tryptose Phosphate Broth is an infusion-free buffered medium recommended for the cultivation of fastidious, pathogenic microorganisms. It can be used in a procedure for the serodiagnosis of *Listeria monocytogenes*.[1] It is valuable in tissue culture procedures,[2] where the peptone content is considered to be a stimulating factor for cells.

Principles of the Procedure

Peptone provides carbon and nitrogen. Dextrose is a carbon source. Sodium chloride maintains osmotic balance. Buffering capacity is provided by disodium phosphate.

The addition of 0.1-0.2% agar to Tryptose Phosphate Broth facilitates anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment.[3] The low agar concentration provides suitable conditions for both aerobic growth in the upper zone and for microaerophilic and anaerobic growth in the lower zone.

Formula

Bacto™ Tryptose Phosphate Broth

Approximate Formula* Per Liter

Tryptose	20.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	g
Disodium Phosphate	2.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 29.5 g of the powder in 1 L of purified water. (If a medium containing 0.1-0.2% agar is desired, add 1-2 g of agar; heat with frequent agitation and boil for 1 minute to completely dissolve the powder.)
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

User Quality Control

Identity Specifications

Bacto™ Tryptose Phosphate Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.95% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent, may have a very slight precipitate.
Prepared Appearance:	Light amber, clear to very slightly opalescent, may have a very slight precipitate.
Reaction of 2.95% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Bacto™ Tryptose Phosphate Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	10^2-10^3	Good
Staphylococcus epidermidis	12228	10^2-10^3	Good
Streptococcus pneumoniae	6305	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

Uninoculated Tube

Streptococcus pneumoniae ATCC™ 6305

Expected Results
Refer to appropriate references and procedures for results.

References

1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. Ginsberg, Gold and Jordan. 1955. Proc. Soc. Exp. Biol. Med. *89*:66.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability
Bacto™ Tryptose Phosphate Broth

BAM

Cat. No.	260300	Dehydrated – 500 g
	260100	Dehydrated – 2 kg
	260200	Dehydrated – 10 kg

Tween™ 80 Water

Intended Use
Tween™* 80 Water may be used to restore and/or inoculate microdilution panels.

**Tween is a trademark of ICI Americas.*

Summary and Explanation
Tween 80 (polysorbate 80) is a surface active agent that is recommended for use at a 0.02% concentration in routine inoculum preparation and dispensing procedures in various microdilution systems.[1]

Principles of the Procedure
Tween 80 Water is a 0.02% concentration of polysorbate 80 in purified water that is convenient for use in dispersing microorganisms during inoculum preparation and for reconstituting antimicrobial agents in microdilution plates.

Procedure
Follow those procedures or test methods requiring the use of water with 0.02% polysorbate 80.

Reference

1. Thrupp. 1986. *In* Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. Williams & Wilkins, Baltimore, Md.

Availability
BBL™ Tween™ 80 Water

Cat. No.	297381	Prepared Tubes (D Tubes), 12.5 mL – Ctn. of 100
	296184	Prepared Tubes (A Tubes), 25 mL – Ctn. of 100

Tyrosine Agar
(See Nocardia Differentiation Media)

UVM Modified Listeria Enrichment Broth

Intended Use

UVM Modified Listeria Enrichment Broth is used for rapidly isolating *Listeria monocytogenes*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985,[3] and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[6]

Listeria species grow over a pH range of 4.4-9.6 and survive in food products with pH levels outside these parameters.[7] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

User Quality Control

Identity Specifications

Difco™ UVM Modified Listeria Enrichment Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is light to medium amber with a faint bluish-green ring at the surface, clear to very slightly opalescent with a fine precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent with a fine precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ UVM Modified Listeria Enrichment Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	10^3-2×10^3	Suppressed at 18-24 hours; good at 40-48 hours
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition
Listeria monocytogenes	19114	10^2-10^3	Good

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

UVM Modified Listeria Enrichment Broth is a modification of the formula described by Donnelly and Baigent.[9] It is used for selective enrichment of *Listeria* spp. from food[7,10] and clinical specimens.[11]

Principles of the Procedure

Peptones, beef extract and yeast extract in UVM Modified Listeria Enrichment Broth provide nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. Phosphate acts as a buffering agent. Nalidixic acid inhibits growth of gram-negative organisms. Acriflavine hydrochloride inhibits many gram-positive bacteria. Esculin is hydrolyzed by *Listeria* species.

Formula

Difco™ UVM Modified Listeria Enrichment Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	5.0	g
Beef Extract	5.0	g
Yeast Extract	5.0	g
Sodium Chloride	20.0	g
Disodium Phosphate	9.6	g
Monopotassium Phosphate	1.35	g
Esculin	1.0	g
Nalidixic Acid	0.02	g
Acriflavine HCl	12.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The USDA method[11] involves enrichment of the specimen in UVM Modified Listeria Enrichment Broth (one part sample in nine parts broth) at 30°C. After incubation, a portion of the enrichment mixture is added to an enrichment broth or plated onto the final isolation agar.[7] For further information when testing food samples or clinical specimens, refer to appropriate references.[7,10-12]

Expected Results
Refer to appropriate references and procedures for results.

References

1. Murray, Webb and Swann. 1926. J. Pathol. Bacteriol. 29:407.
2. Monk, Clavero, Beuchat, Doyle and Brackett. 1994. J. Food Prot. 57:969.
3. Wehr. 1987. J. Assoc. Off. Anal. Chem. 70:769.
4. Bremer and Osborne. 1995. J. Food Prot. 58:604.
5. Grau and Vanderlinde. 1992. J. Food Prot. 55:4.
6. Patel, Hwang, Beuchat, Doyle and Brackett. 1995. J. Food Prot. 58:244.
7. Ryser and Donnelly. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
8. Kramer and Jones. 1969. J. Appl. Bacteriol. 32:381.
9. Donnelly and Baigent. 1986. Appl. Environ. Microbiol. 52:689.
10. McClain and Lee. May 24, 1989. Laboratory communication No. 57. Microbiology Division, Food Safety and Inspection Service, U.S. Department of Agriculture, Beltsville, Md.
11. Bille, Rocourt and Swaminathan. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
12. Hayes, Graves, Swaminathan, Ajello, Marcolm, Weaver, Ransom, Deaver, Plikaytis, Schuchat, Wenger, Pinner, Broome and The *Listeria* Study Group. 1992. J. Food. Prot. 55:952.

Availability
Difco™ UVM Modified Listeria Enrichment Broth

AOAC CCAM COMPF USDA

Cat. No.	222330	Dehydrated – 500 g
	222310	Dehydrated – 2 kg
	222320	Dehydrated – 10 kg

Universal Beer Agar

Intended Use
Universal Beer Agar (UBA Medium) is used for cultivating microorganisms of significance in the brewing industry.

Summary and Explanation
Universal Beer Agar is a basal medium to which beer is added. It is based on the formula developed by Kozulis and Page[1] who compared it with other media commonly used in breweries for detecting microbial contamination.[2] The characteristics of Universal Beer Agar are closer to the natural environmental conditions found in the typical brewery than other media studied. It supports growth of more varieties of lactic acid bacteria and yields larger colonies in a shorter time than traditional brewer's media. Due to the presence of beer in the medium, it is selective for growth of microorganisms that have adapted themselves to existent conditions in the brewery. The presence of hop constituents and alcohol inhibits growth of many airborne microorganisms not adapted to this environment.[3]

Universal Beer Agar supports growth of *Lactobacillus*, *Pediococcus*, *Acetobacter* and yeast strains which may be found contaminating the wort and beer.

User Quality Control

Identity Specifications
Difco™ Universal Beer Agar

Dehydrated Appearance:	Medium beige, homogeneous, free-flowing.
Solution:	6.2% solution, soluble in purified water upon boiling. Solution is medium to dark amber, very slightly opalescent.
Prepared Appearance:	Medium to dark amber, very slightly opalescent.
Reaction of 6.2% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response
Difco™ Universal Beer Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 3 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Acetobacter pasteurianus	12879	10^2-10^3	Good
Lactobacillus fermentum	9338	10^2-10^3	Good
Lactobacillus johnsonii	11506	10^2-10^3	Good
Pediococcus acidilactici	8081	10^2-10^3	Good

Principles of the Procedure
Yeast extract is a source of trace elements, vitamins and amino acids. Peptonized milk contains lactose as an energy source. Tomato juice is a source of carbon, protein and nutrients. Dextrose provides additional carbon. Dipotassium and monopotassium phosphates provide buffering capability. Magnesium sulfate, ferrous sulfate and manganese sulfate are sources of ions that stimulate metabolism. Sodium chloride provides essential ions. Agar is the solidifying agent.

Formula
Difco™ Universal Beer Agar

Approximate Formula* Per Liter		
Yeast Extract	6.1	g
Peptonized Milk	15.0	g
Tomato Juice (from 244 mL)	12.2	g
Dextrose	16.1	g
Dipotassium Phosphate	0.31	g
Monopotassium Phosphate	0.31	g
Magnesium Sulfate	0.12	g
Sodium Chloride	6.0	mg
Ferrous Sulfate	6.0	mg
Manganese Sulfate	6.0	mg
Agar	12.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 62 g of the powder in 750 mL of purified water (or halogen-free tap water). Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. While the medium is still hot, add 250 mL commercial beer (not degassed) and mix well.

4. Autoclave at 121°C for 10 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
See appropriate references for specific procedures.

Expected Results
Refer to appropriate references and procedures for results.

References
1. Kozulis and Page. 1968. Proc. Am. Soc. Brewing Chemists, p. 52.
2. Murphy and Saletan. 1970. Tech. Q. Master Brew. Assoc. Am. 7:182.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability
Difco™ Universal Beer Agar

Cat. No. 285610 Dehydrated – 500 g

Mexico

Cat. No. 252644 Prepared Plates (60 × 15 mm-style) – Pkg. of 20*
252645 Prepared Flasks, 140 mL

**Store at 2-8°C.*

Universal Preenrichment Broth

Intended Use
Universal Preenrichment Broth is used for recovering sublethally injured *Salmonella* and *Listeria* from food products.

Summary and Explanation
Traditional methods for recovering *Salmonella* and *Listeria* from food products require separate preenrichment media for each microorganism.[1, 2] Some broth media recommended for preenrichment contain antibiotic inhibitors[3] or have insufficient buffering capacity which hinder recovery of sublethally injured cells.[3-5]

Bailey and Cox[3] formulated Universal Preenrichment Broth to permit simultaneous resuscitation of sublethally injured *Salmonella* and *Listeria*. The broth medium provides sufficient buffering capacity to prevent rapid decreases in pH and allows for repair of injured cells that might be sensitive to low pH values or inhibitory substances.

Principles of the Procedure
Universal Preenrichment Broth contains peptones as sources of carbon, nitrogen, vitamins and minerals. Sodium and potassium phosphates buffer the medium. Sodium chloride maintains the osmotic balance of the medium. Magnesium sulfate and ferric ammonium citrate provide essential ions. Dextrose is an energy source. Sodium pyruvate helps stimulate the metabolism of stressed organisms.

User Quality Control

Identity Specifications
Difco™ Universal Preenrichment Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water. Solution is light to medium amber, slightly opalescent to opalescent, may have a precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent to opalescent, may have a precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response
Difco™ Universal Preenrichment Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Listeria monocytogenes	19115	10-10^2	Good
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10-10^2	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10-10^2	Good

Uninoculated Tube

Salmonella Typhimurium ATCC™ 14028

Formula

Difco™ Universal Preenrichment Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone	5.0	g
Monopotassium Phosphate	15.0	g
Disodium Phosphate	7.0	g
Sodium Chloride	5.0	g
Dextrose	0.5	g
Magnesium Sulfate	0.25	g
Ferric Ammonium Citrate	0.1	g
Sodium Pyruvate	0.2	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 38 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Procedures for the preenrichment of *Salmonella* and *Listeria* are provided in appropriate references.[1,2]

Expected Results

Salmonella and *Listeria* demonstrate good growth and recovery following preenrichment in this broth.

References

1. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
3. Bailey and Cox. 1992. J. Food Prot. *55*:256.
4. Bailey, Fletcher and Cox. 1990. J. Food Prot. *53*:473.
5. Juven, Cox, Bailey, Thomson, Charles and Shutze. 1984. J. Food Prot. *47*:299.

Availability

Difco™ Universal Preenrichment Broth

CCAM

Cat. No. 223510 Dehydrated – 500 g

Urea Media
Urea Agar Base • Urea Agar Base Concentrate 10×
Urea Agar • Urea Broth • Urease Test Broth
Urease Broth Concentrate 10×

Intended Use

Urea Agar and Urease Test Broth are used for the differentiation of organisms, especially the *Enterobacteriaceae*, on the basis of urease production.

Summary and Explanation

Urea Agar was devised by Christensen for use as a solid medium for the differentiation of enteric bacilli.[1] It differentiates between rapid urease-positive *Proteeae* organisms (*Proteus* spp., *Morganella morganii* subsp. *morganii, Providencia rettgeri*, and some *Providencia stuartii*) and other urease-positive organisms: *Citrobacter, Enterobacter* and *Klebsiella* and bacteria other than *Enterobacteriaceae*; i.e., some *Bordetella* and *Brucella* spp.[2]

The base is also supplied as a filter-sterilized 10× concentrated solution in tubes for use in preparing Urea Agar slants in the laboratory.

Urease Test Broth was developed by Rustigian and Stuart.[3] It may be used for the identification of bacteria on the basis of urea utilization and it is particularly recommended for the differentiation of members of the genus *Proteus* from those of *Salmonella* and *Shigella* in the diagnosis of enteric infections.[4] The medium is positive for *Proteus*, *Morganella morganii* subsp. *morganii*, *Providencia rettgeri*, and a few *Providencia stuartii* strains with the reclassification of the members of the *Proteeae*.

Urease base is also supplied as a filter sterilized 10× concentrated solution for use in preparing Urease Test Broth in the laboratory.

Principles of the Procedure

The urea medium of Rustigian and Stuart[3] is particularly suited for the differentiation of *Proteus* species from other gram-negative enteric bacilli capable of utilizing urea;[1] the latter are unable to do so in Urease Test Broth because of limited nutrients and the high buffering capacity of the medium. To provide a medium with greater utility, Urea Agar was devised by Christensen[1] with peptone and dextrose included and reduced buffer content to promote more rapid growth of many of the *Enterobacteriaceae* and permit a reduction in incubation time. The complete Urea Agar contains 15.0 g/L of agar in addition to the ingredients in the base medium.

When organisms utilize urea, ammonia is formed during incubation which makes the reaction of these media alkaline, producing a red-pink color. Consequently, urease production may be detected by the change in the phenol red indicator.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Urea Broth

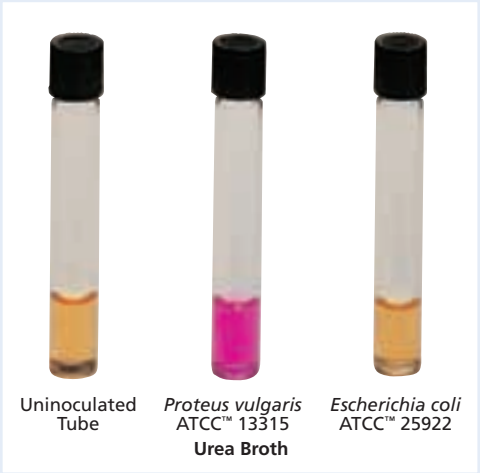
Dehydrated Appearance:	Light orange to light pink, homogeneous, inherently lumpy.
Solution:	3.87% solution, soluble in purified water. Solution is orange-yellow, clear.
Prepared Appearance:	Orange-yellow, clear.
Reaction of 3.87% Solution at 25°C:	pH 6.8 ± 0.1

Cultural Response

Difco™ Urea Broth

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 8-48 hours.

ORGANISM	ATCC™	UREASE REACTION
Enterobacter aerogenes	13048	–
Escherichia coli	25922	–
Proteus mirabilis	25933	+
Proteus vulgaris	13315	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	–



Uninoculated Tube | *Proteus vulgaris* ATCC™ 13315 | *Escherichia coli* ATCC™ 25922

Urea Broth

Identity Specifications

BBL™ Urea Agar Base

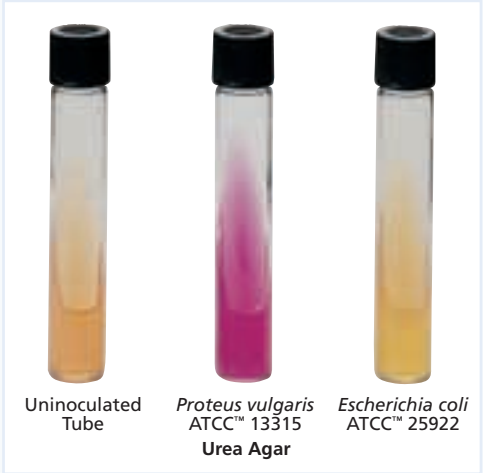
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	29 g/100 mL solution, soluble in purified water. Complete medium is light to medium, orange, clear to slightly hazy.
Prepared Appearance:	Complete medium is light to medium, orange, clear to slightly hazy.
Reaction of 2.9% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Urea Agar Base

Prepare the medium per label directions. Inoculate with fresh cultures (2 heavy loopfuls) and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	UREASE REACTION
Proteus vulgaris	8427	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	13311	–



Uninoculated Tube | *Proteus vulgaris* ATCC™ 13315 | *Escherichia coli* ATCC™ 25922

Urea Agar

Formulae

BBL™ Urea Agar Base

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	1.0	g
Dextrose	1.0	g
Sodium Chloride	5.0	g
Potassium Phosphate	2.0	g
Urea	20.0	g
Phenol Red	12.0	mg

Difco™ Urea Broth

Approximate Formula* Per Liter

Yeast Extract	0.1	g
Monopotassium Phosphate	9.1	g
Dipotassium Phosphate	9.5	g
Urea	20.0	g
Phenol Red	0.01	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

BBL™ Urea Agar Base

1. Dissolve 29 g of the powder in 100 mL of purified water. Mix thoroughly. Sterilize by filtration.
2. Suspend 15 g of agar in 900 mL of purified water.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and add 100 mL of the sterile Urea Agar Base.
5. Mix thoroughly and dispense aseptically in sterile tubes.
6. Cool tubed medium in a slanted position so that deep butts are formed.
7. Do not remelt the complete medium.
8. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Urea Agar Base Concentrate 10× (Prepared Tubes)

1. To prepare Urea Agar medium, add 1.7 g of granulated agar to 100 mL of purified water. Heat with agitation and boil for 1 minute.
2. Dispense in 9 mL aliquots into tubes and autoclave at 121°C for 15 minutes.
3. Cool the agar to 45-50°C, and allow one tube of concentrate to come to room temperature. Add 1 mL of concentrate to each 9 mL of cooled agar solution and mix thoroughly.
4. Allow the tubes to cool in a slanted position so that slants with deep butts are formed.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Urea Broth

1. Equilibrate the medium to room temperature before opening. The presence of urea in this medium renders it inherently lumpy. This condition will not adversely affect a properly stored medium.
2. Dissolve 38.7 g of the powder in 1 L of purified water. Mix thoroughly to completely dissolve the powder.
3. Filter sterilize. DO NOT BOIL OR AUTOCLAVE THE MEDIUM.
4. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Urease Broth Concentrate 10× (Prepared Tubes)

1. To prepare medium, aseptically add 10 mL of the concentrate to 90 mL of cold sterile purified water. Mix thoroughly.
2. Dispense aseptically in 1-3 mL amounts, in small sterile test tubes.

Procedure

If Urea Agar Base Concentrate 10× or Urease Broth Concentrate 10× is being used, prepare the complete medium as described above. If crystals form in the concentrate, they will usually dissolve at room temperature, or in a few minutes in a 40°C water bath.

Using a heavy inoculum (2 loopfuls) of growth from an 18- to 24-hour pure culture (TSI Agar or other suitable medium), inoculate the broth or agar (streaking back and forth over the entire slant surface). Do not stab the butt since it serves as a color control. For broth, shake tubes gently to suspend the bacteria. Incubate tubes with loosened caps at 35 ± 2°C in an incubator or water bath. Observe reactions after 2, 4, 6, 18, 24 and 48 hours. For agar, continue to check every day for a total of 6 days; even longer incubation periods may be necessary.

Expected Results

The production of urease is indicated by an intense pink-red (red-violet) color on the slant or throughout the broth. The color may penetrate into the agar (butt); the extent of the color indicates the rate of urea hydrolysis.[5]

A negative reaction is no color change. The agar medium remains pale yellow to buff; the broth remains yellowish-orange.

For a listing of urease-positive organisms, consult appropriate texts.[2, 4-7]

Limitations of the Procedure

Urea Agar Base

1. The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. False positive reactions may occur due to the utilization of peptones (especially in slant agar by *Pseudomonas aeruginosa*, for example) or other proteins which raise the pH due to protein hydrolysis and the release of excessive amino acid residues. To eliminate possible protein hydrolysis, perform a control test with the same test medium without urea.[7]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. Urea Agar detects rapid urease activity of only the urease-positive *Proteus* species. For results to be valid for the detection of *Proteus*, the results must be read within the first 2-6 hours after incubation. Urease-positive *Enterobacter*, *Citrobacter* or *Klebsiella*, in contrast, hydrolyze urea much more slowly, showing only slight penetration of the alkaline reaction into the butt of the medium in 6 hours and requiring 3-5 days to change the reaction of the entire butt.

Urea Broth

1. To rule out false positives due to protein hydrolysis (as opposed to urea hydrolysis) that may occur in the medium after prolonged incubation, perform a control test with the same test medium without urea.[7]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. The high buffering system in this medium masks urease activity in organisms that are delayed positive. This medium is therefore recommended for the detection of urease activity in all *Proteus* spp., *Providencia rettgeri* and urease-positive *Providencia stuartii*.[1] *M. morganii* slowly hydrolyzes urea and may require approximately a 36 hour incubation for a strong urease-positive reaction to occur.[1] If in doubt as to a result, compare with an uninoculated tube or incubate for an additional 24 hours.
4. Variations in the size of the inoculum can affect the time required to reach positive (alkaline, pH 8.1) results.

References

1. Christensen. 1946. J. Bacteriol. *52*:461.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Rustigian and Stuart. 1941. Proc. Soc. Exp. Biol. Med. *47*:108.
4. Ewing. 1985. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co, Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Urea Agar Base

BAM CCAM ISO USDA

Cat. No. 211795 Dehydrated – 500 g*

BBL™ Urea Agar Base Concentrate 10×

Cat. No. 221100 Prepared Tubes – Pkg. of 10*

BBL™ Urea Agar

Cat. No. 221096 Prepared Slants – Pkg. of 10*
221097 Prepared Slants – Ctn. of 100*

Difco™ Urea Broth

AOAC BAM COMPF SMD

Cat. No. 227210 Dehydrated – 500 g*

BBL™ Urease Test Broth

AOAC BAM COMPF SMD

Cat. No. 221719 Prepared Tubes, 3 mL – Pkg. of 10*

BBL™ Urease Broth Concentrate 10×

Cat. No. 221098 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*

V Agar

Intended Use

V Agar is an enriched medium used in qualitative procedures for the isolation and differentiation of *Gardnerella vaginalis* from clinical specimens.

Summary and Explanation

In 1966, Ellner et al. developed an improved blood agar base formulation, which has been designated as Columbia Agar.[1]

Greenwood et al., in 1977, described a modification of Columbia Agar in which the peptone concentration was increased and human blood was used.[2] This enriched medium was designed for the isolation and differentiation of *G. vaginalis* by means of beta hemolysis of human blood.[3,4] Greenwood et al. reported that 96% of *G. vaginalis* isolated produced beta hemolysis of human blood, whereas none were beta-hemolytic on sheep blood.[5]

Principles of the Procedure

V Agar contains peptones, beef extract and yeast extract, which supply the nutrients required for the growth of *G. vaginalis* strains. The peptones and beef extract are sources of nitrogenous compounds, carbon, sulfur and trace ingredients. The yeast extract and corn starch serve as energy sources with the yeast extract being a supplier of the B-complex vitamins.

The human blood aids in the identification of *G. vaginalis* since the small size of the colonies and the diffuse hemolysis is distinctive compared to other hemolytic colonies.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Since *G. vaginalis* requires carbon dioxide on primary isolation, plates should be incubated in an aerobic atmosphere containing approximately 3-10% CO_2 at 35 ± 2°C for 48 hours.[6]

Expected Results

Typical colonies of *G. vaginalis* appear small and white, yield gram-variable diphtheroid-like forms and exhibit distinctive diffuse beta hemolysis after 48 hours of incubation in an aerobic atmosphere supplemented with carbon dioxide.

References

1. Ellner, Stoessel, Drakeford and Vasi. 1966. Am. J. Clin. Pathol. *45*:502.
2. Greenwood, Pickett, Martin and Mack. 1977. Health Lab Sci. *14*:102.
3. Greenwood and Pickett. 1980. Int. J. Syst. Bacteriol. *30*:170.
4. Piot, Van Dyck, Goodfellow and Falkow. 1980. J. Gen. Microbiol. *119*:373.
5. Greenwood and Pickett. 1979. J. Clin. Microbiol. *9*:200.
6. Funke and Bernard. 2007. *In* Murray, Baron, Jorgensen, Landry and Pfaller (ed), Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ V Agar

CMPH2 MCM9

United States and Canada

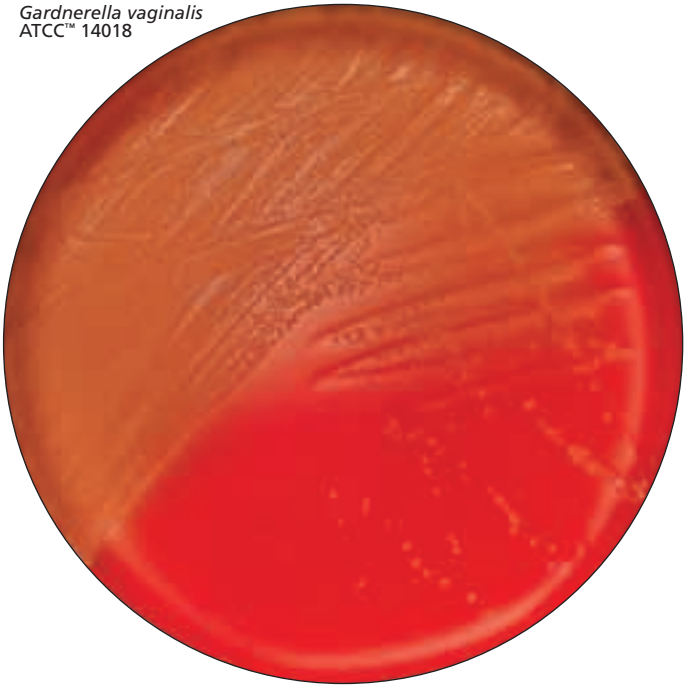
Cat. No. 221874 Prepared Plates – Pkg. of 10*
221875 Prepared Plates – Ctn. of 100*

Mexico

Cat. No. 221874 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Gardnerella vaginalis
ATCC™ 14018



VJ Agar (Vogel and Johnson Agar)

Intended Use

VJ Agar, also known as Vogel and Johnson Agar, is used for the early detection of coagulase-positive, mannitol-fermenting staphylococci.

Summary and Explanation

In 1955, Zebovitz et al. developed Tellurite-Glycine Agar as a selective plating medium for the quantitative detection of coagulase-positive staphylococci.[1] This medium was modified by Vogel and Johnson in 1960 by the addition of phenol red as a pH indicator and by increasing the mannitol content.[2] VJ Agar selects and differentiates the coagulase-positive staphylococci which ferment mannitol and reduce tellurite.[3]

VJ Agar is specified as a standard methods medium for cosmetics,[4,5] pharmaceutical articles[6] and nutritional supplements.[6]

Principles of the Procedure

Peptone is a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Mannitol is the carbohydrate. Inhibition of nonstaphylococcal organisms is achieved by the potassium tellurite additive, which is inhibitory for some species of both gram-positive and gram-negative bacteria, by lithium chloride and by the high glycine content. Staphylococci may be slightly inhibited by the presence of the three inhibitors; however, this is compensated for by the addition of mannitol and glycine.[3] Phenol red is the pH indicator and agar is the solidifying agent.

Coagulase-positive staphylococci reduce the potassium tellurite to metallic free tellurium, producing colonies that are gray-black. The fermentation of mannitol by coagulase-positive staphylococci is detected by a change in the color of the phenol red indicator from red (alkaline) to yellow (acid).

Prepared plates of Vogel and Johnson Agar contain 0.2 g/L of potassium tellurite.

Formula

Difco™ VJ Agar

Approximate Formula* Per Liter		
Tryptone	10.0	g
Yeast Extract	5.0	g
Mannitol	10.0	g
Dipostassium Phosphate	5.0	g
Lithium Chloride	5.0	g
Glycine	10.0	g
Agar	15.0	g
Phenol Red	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ VJ Agar

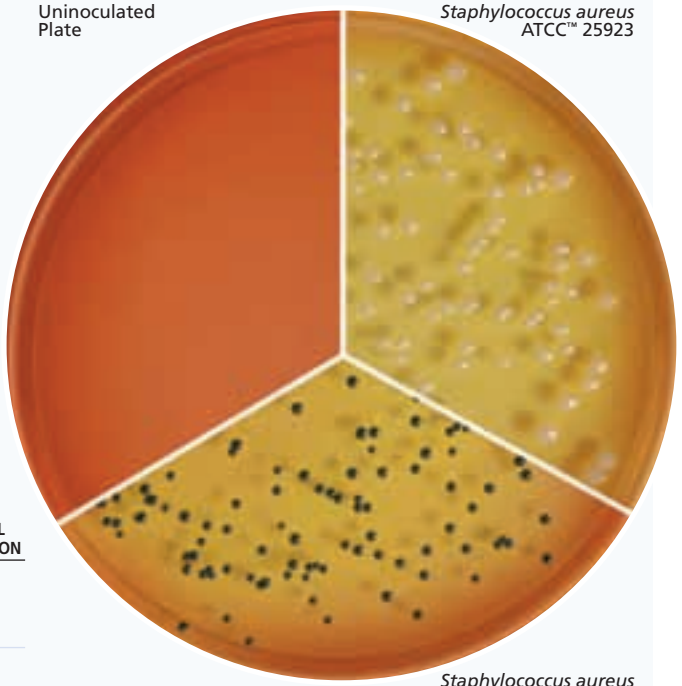
Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in purified water upon boiling. Solution is red, very slightly to slightly opalescent, may have a slight white precipitate.
Prepared Appearance:	Red, slightly opalescent, may have a slight white precipitate.
Reaction of 6.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ VJ Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	TELLURITE REDUCTION	MANNITOL FERMENTATION
Escherichia coli	25922	3×10^2-10^3	Marked to complete inhibition	– (translucent)	– (red)
Proteus mirabilis	25933	3×10^2-10^3	Partial to complete inhibition	+ (black)	– (red)
Staphylococcus aureus	25923	10^2-3×10^2	Good	+ (black)	+ (yellow)
Staphylococcus epidermidis	12228	3×10^2-10^3	Fair to good	± (translucent to black)	– (red)



Directions for Preparation from Dehydrated Product

1. Suspend 60 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Add 20 mL of sterile Tellurite Solution 1%. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates for 18-48 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

After incubation, examine the isolated colonies on the plated medium. During the first 18-24 hours of incubation, most organisms, other than coagulase-positive staphylococci, are totally or markedly inhibited. By 48 hours, many coagulase-negative, mannitol-fermenting or coagulase-negative, mannitol-negative staphylococci will appear on the medium.

The coagulase-positive cocci form small, black colonies on red plates. If mannitol is fermented, the colonies are surrounded by yellow zones due to the color change of the phenol red indicator in response to the acid formation. If mannitol has not been fermented, no yellow zone is present, and the color of the medium around the colonies may even be a deeper red than normal due to utilization of the peptones in the medium.

References

1. Zebovitz, Evans and Niven. 1955. J. Bacteriol. *70*:686.
2. Vogel and Johnson. 1960. Public Health Lab. *18*:131.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Hitchins, Tran and McCarron. 2001. *In* FDA bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
5. Curry, Graf and McEwen (ed.). 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
6. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ VJ Agar

BAM

Cat. No. 256220 Dehydrated – 500 g

BBL™ Vogel and Johnson Agar

BAM

Cat. No. 298220 Prepared Plates – Pkg. of 10*

BBL™ Tellurite Solution 1%

Cat. No. 211917 Tube – 20 mL*

**Store at 2-8°C.*

Vancomycin Screen Agar

Intended Use

Vancomycin Screen Agar is used to test enterococci for resistance to vancomycin and to predict the synergistic activity of this antimicrobial with an aminoglycoside antimicrobial.

Summary and Explanation

Enterococci are known to cause a wide variety of infections. Most commonly they infect the urinary tract, abdomen, bloodstream, endocardium, biliary tract, burn wounds and in-dwelling catheters.[1] *Enterococcus faecalis* causes 80 to 90% of infections, while *E. faecium* causes the majority of the remainder.[2] Today the enterococci are the fourth leading cause of hospital acquired infection and the third leading cause of bacteremia in the United States.[3] The case/fatality rates for enterococcal bacteremia range from 12 to 68% with death due to sepsis in 4 to 50% of the cases.[4]

Treatment of enterococcal infections with either penicillin or vancomycin alone fails to kill enterococci resulting in relapse of infection.[5] Enterococci for years were known to have low intrinsic resistance to a variety of β-lactam as well as aminoglycoside antibiotics.[6] The addition of an aminoglycoside to which the isolate has demonstrated susceptibility results in both *in vitro* and *in vivo* synergism producing a bactericidal effect.[7] This synergistic effect is thought to be due to the penicillin or vancomycin damaging the integrity of the cell

Enterococcus faecalis
ATCC™ 51299

wall, thus allowing the aminoglycoside to penetrate and inhibit bacterial protein synthesis.[8] The emergence of resistance to vancomycin (≥ 6 µg/mL)[9] may result in the failure of vancomycin-aminoglycoside combinations to eradicate the infecting organisms. Therefore, testing for resistance to vancomycin is important. The use of a Brain Heart Infusion Agar (BHIA) containing vancomycin (6 µg/mL) is recommended by the Clinical and Laboratory Standards Institute (CLSI) for testing resistance.[10, 11]

Principles of the Procedure

The Brain Heart Infusion Agar base is a general-purpose medium suitable for the cultivation of a wide variety of microorganisms.

The meat infusion solids and peptones are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is a carbohydrate source. The medium is buffered through the use of disodium phosphate. Vancomycin at 6 µg/mL is used to detect resistance to vancomycin.[11] The Food, Drug & Cosmetic (FD & C) dye is inert and added for easy visual identification of this vancomycin-containing medium.

Procedure

1. Prepare the inoculum by suspending several well-isolated colonies of the enterococcal isolate from an 18-24 hour plate culture into a tube of **Trypticase**™ Soy Broth and adjust the turbidity to be equivalent to a 0.5 McFarland turbidity standard.
2. Spot inoculate the plate with 10 µL of the adjusted suspension.[12]
3. Allow the inoculum spot to absorb into the agar surface.
4. Inoculate a growth control plate, such as a **Trypticase** Soy Agar with 5% Sheep Blood, in the same manner.
5. Incubate plates at 35 ± 2°C aerobically for a full 24 hours.

Expected Results

Following a full 24 hours of incubation, observe plates for growth.

Growth control plate: Growth indicates viable test organisms in the inoculum broth suspension and the test is valid. If there is no growth, the test is invalid and must be repeated.

BHIA with vancomycin (Yellow): Growth (> one colony) indicates that the antimicrobial agent may not be synergistic in combination therapy. No growth (≤ one colony) indicates that synergy may be predicted.[11]

References

1. Jett, Huycke and Gilmore. 1994. Clin. Microbiol. Rev. 7:462.
2. Moellering. 1992. Clin. Infect. Dis. *14*:1173.
3. Emori and Gaynes. 1993. Clin. Microbiol. Rev. 6:428.
4. Landry, Kaiser and Wenzel. 1989. Am. J. Infect. Control. *17*:323.
5. Moellering, Korzeniowski, Sande and Wennersten. 1979. J. Infect. Dis. *140*:203.
6. Murray. 1990. Clin. Microbiol. Rev. *3*: 46.
7. Mandell. 1984. Ann. Intern. Med. *100*:904.
8. Moellering and Weinberg. 1971. J. Clin. Invest. *50*:2580.
9. Johnson, Uttley, Woodford and George. 1990. Clin. Microbiol. Rev. 3:280.
10. Clinical and Laboratory Standards Institute. 2006. Approved standard: M7-A7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. CLSI, Wayne, Pa.
11. Clinical and Laboratory Standards Institute. 2008. M100-S18 (M7). MIC testing supplemental tables. CLSI, Wayne, Pa.
12. Sahm and Torres. 1988. J. Clin. Microbiol. 26:250.

Availability

BBL™ Vancomycin Screen Agar

BS12 CLSI CMPH2 MCM9

Cat. No. 222204 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*

Veal Infusion Agar • Veal Infusion Broth

Intended Use

Veal Infusion Agar is used for cultivating fastidious microorganisms with or without added enrichment.

Veal Infusion Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

The nutritive factors of veal infusion media permit luxuriant growth of fastidious microorganisms. Veal Infusion Agar may be used as a base with blood, ascitic fluid, serum or other enrichments. Veal infusion media are specified for use in the examination of food.[1,2] Veal Infusion Agar is specified in *Official Methods of Analysis of AOAC International* for culturing eggs and egg products, and as a maintenance medium for *E. coli*.[3] Veal Infusion Broth is recommended for culturing *E. coli* in the AOAC procedure for invasiveness of mammalian cells.[3]

Principles of the Procedure

Infusion from lean veal and peptone provide the nitrogen, vitamins, carbon and amino acids in veal infusion media. Sodium chloride maintains the osmotic balance of the formulations. Agar is the solidifying agent in Veal Infusion Agar.

Formulae

Difco™ Veal Infusion Agar

Approximate Formula* Per Liter

Lean Veal, Infusion from 500 g	10.0	g
Proteose Peptone No. 3	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

Difco™ Veal Infusion Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ Veal Infusion Agar

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.0% Solution at 25°C:	pH 7.4 ± 0.2

Difco™ Veal Infusion Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.5% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent with no more than very slight precipitation.
Reaction of 2.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Veal Infusion Agar or Veal Infusion Broth

Prepare the agar medium per label directions without (plain) and with 5% sterile defibrinated sheep blood (SB). Inoculate and incubate plates at 35 ± 2°C for 18-48 hours under approximately 10% CO_2.

Prepare the broth medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AGAR (PLAIN)/BROTH	RECOVERY AGAR WITH SB
Neisseria meningitidis	13090	10^2-10^3	Good	Good
Staphylococcus epidermidis	12228	10^2-10^3	Good	Good
Streptococcus mitis	9895	10^2-10^3	Good	Good
Streptococcus pneumoniae	6305	10^2-10^3	Good	Good

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Veal Infusion Agar – 40 g;
   Difco™ Veal Infusion Broth – 25 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool the agar medium to 45-50°C and aseptically add sterile serum, defibrinated blood or other enrichment, as desired. Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on the examination of fastidious microorganisms in food refer to the procedures outlined in the references.[1-3]

Expected Results

Refer to appropriate references and procedures for results.

References

1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Veal Infusion Agar

AOAC BAM COMPF

Cat. No. 234310 Dehydrated – 500 g

Difco™ Veal Infusion Broth

AOAC BAM COMPF

Cat. No. 234420 Dehydrated – 500 g
234410 Dehydrated – 10 kg

Violet Red Bile Agar

Intended Use

Violet Red Bile Agar is used for enumerating coliform organisms in dairy products.

Summary and Explanation

The coliform group of bacteria includes aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Members of the *Enterobacteriaceae* comprise the majority of the group but other lactose fermenting organisms may also be included.

Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products.[1-3] One method for performing the presumptive test for coliforms uses Violet Red Bile Agar. If typical coliform colonies appear, they are tested further to confirm their identification as coliforms.

Principles of the Procedure

Violet Red Bile Agar contains peptone to provide carbon and nitrogen sources for general growth requirements. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Bile salts and crystal violet inhibit most gram-positive microorganisms. Lactose is the carbohydrate source and neutral red is the pH indicator. Agar is the solidifying agent.

User Quality Control

Identity Specifications

Difco™ Violet Red Bile Agar

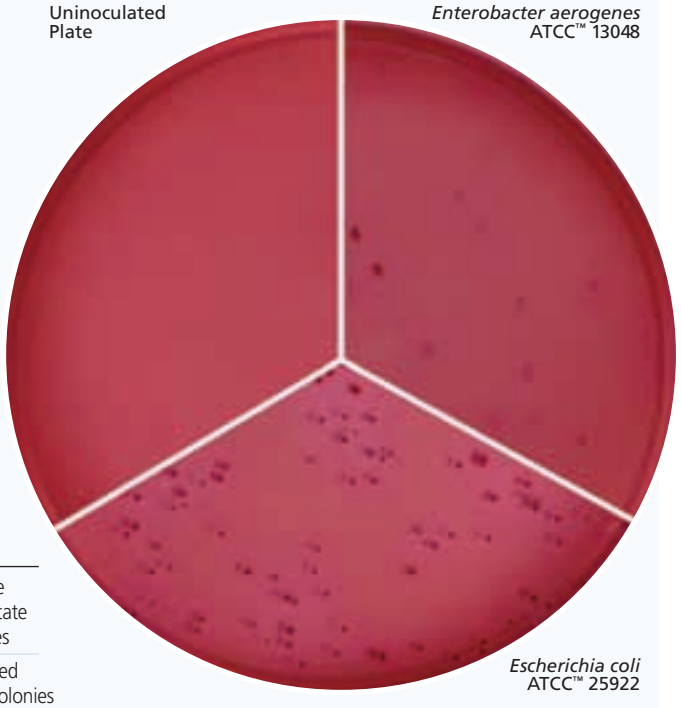
Dehydrated Appearance:	Beige to reddish-beige, homogeneous, free-flowing.
Solution:	4.15% solution, soluble in purified water upon boiling. Solution is reddish-purple, slightly opalescent, without significant precipitate.
Prepared Appearance:	Reddish-purple, slightly opalescent, no significant precipitate.
Reaction of 4.15% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Violet Red Bile Agar

Prepare the medium per label directions. Inoculate and incubate at 32 ± 1°C for 24 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterobacter aerogenes	13048	30-300	Good	Red, may have slight red precipitate around colonies
Escherichia coli	25922	30-300	Good	Deep red with red precipitate around colonies
Staphylococcus aureus	25923	~10^3	Marked to complete inhibition	–



Formula

Difco™ Violet Red Bile Agar

Approximate Formula* Per Liter

Yeast Extract	3.0	g
Peptone	7.0	g
Bile Salts No. 3	1.5	g
Lactose	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Neutral Red	0.03	g
Crystal Violet	2.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 41.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and use immediately.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Presumptive test for coliforms using solid medium:

1. Transfer a 1 mL aliquot of test sample to a Petri dish.
2. Add 10 mL of Violet Red Bile Agar (at 48°C) and swirl to mix.
3. Allow medium to solidify before incubating at 35°C for 18-24 hours; use 32°C for dairy products.
4. Examine for purple-red colonies, 0.5 mm in diameter (or larger), surrounded by a zone of precipitated bile acids.
5. Continue with confirmatory testing of typical coliform colonies.[1-3]

Expected Results

Lactose fermenters: Purple-red colonies, with or without a zone of precipitate around the colonies

Lactose nonfermenters: Colorless to transparent colonies

Gram-positive cocci: Colorless, pinpoint colonies

Limitations of the Procedure

1. Violet Red Bile Agar may not be completely inhibitory to gram-positive organisms. Perform Gram stain and biochemical tests as necessary to identify isolates.
2. The medium will grow gram-negative bacilli other than members of the *Enterobacteriaceae*. Perform biochemical tests to identify isolates to genus and species.
3. Boiling the medium for longer than 2 minutes can decrease the ability to support growth.
4. Plates of Violet Red Bile Agar should not be incubated longer than 24 hours because microorganisms that are only partially inhibited may grow after extended incubation.
5. For optimum performance, prepare and use the medium within 24 hours.

References

1. Davidson, Roth, and Gambrel-Lenarz. 2004. *In* Wehr and Frank (ed.). Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
2. Kornacki and Johnson. 2001. *In* Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Violet Red Bile Agar

BAM CCAM COMPF ISO SMD

Cat. No. 211695 Dehydrated – 500 g
211687 Dehydrated – 2 kg

Mexico
Cat. No. 252633 Prepared Bottles, 140 mL – Pkg. of 12

Violet Red Bile Agar with MUG

Intended Use

Violet Red Bile Agar with MUG is used for enumerating *Escherichia coli* and total coliform bacteria in food and dairy products.

Summary and Explanation

Violet Red Bile Agar is specified in standard methods procedures to enumerate coliforms in food and dairy products.[1-3] In 1982, Feng and Hartman developed a rapid fluorogenic assay for *Escherichia coli* by incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) into Lauryl Tryptose Broth.[4] Incorporating MUG into Violet Red Bile Agar permits the detection of *E. coli* among the coliform colonies.[2, 3]

Standard methods procedures include Violet Red Bile Agar with MUG for detecting *E. coli* in food and dairy products by fluorescence.[1-3]

Principles of the Procedure

Violet Red Bile Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Bile salts and crystal violet inhibit gram-positive bacteria. Lactose is a carbohydrate source. Neutral red is a pH indicator. MUG (4-methylumbelliferyl-β-D-glucuronide) is a substrate used for detecting glucuronidase activity. Agar is the solidifying agent.

E. coli produces the enzyme glucuronidase which hydrolyzes MUG to yield a fluorogenic compound detectable with long-wave UV light (366 nm). Typical strains of *E. coli* (red colonies surrounded by a bile precipitate) exhibit blue fluorescence. Non-*E. coli* coliforms may produce red colonies with zones of precipitated bile but they are MUG negative.

User Quality Control

Identity Specifications

Difco™ Violet Red Bile Agar with MUG

Dehydrated Appearance:	Reddish beige, free-flowing, homogeneous.
Solution:	4.16% solution, soluble in purified water upon boiling. Solution is reddish-purple, clear to slightly opalescent, without significant precipitate.
Prepared Appearance:	Reddish-purple, clear to slightly opalescent, no significant precipitate.
Reaction of 4.16% Solution at 25°C:	pH 7.4 ± 0.2

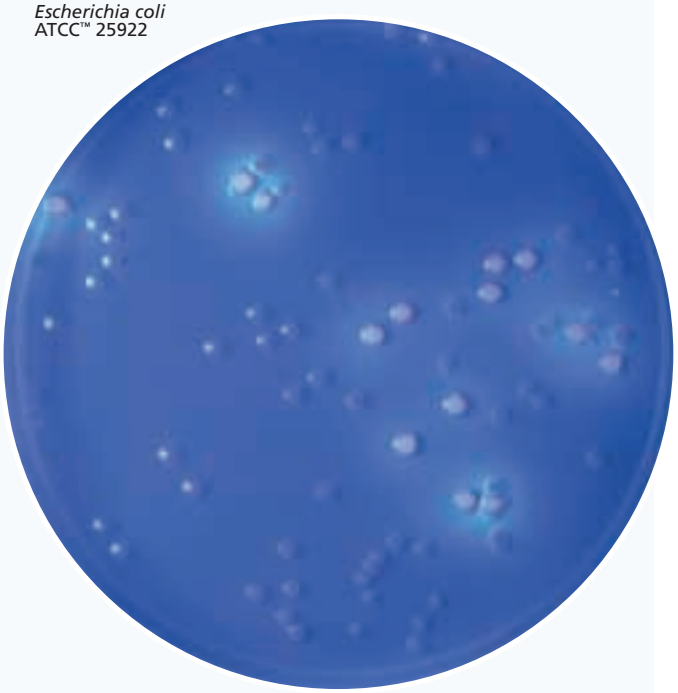
Cultural Response

Difco™ Violet Red Bile Agar with MUG

Prepare the medium per label directions. Inoculate and incubate at 32 ± 2°C for 22-26 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	FLUORESCENCE
Enterobacter aerogenes	13048	30-300	Good	Pink, may have bile ppt.	–
Escherichia coli	25922	30-300	Good	Deep red, with bile ppt.	+
Staphylococcus aureus	25923	3×10^2-10^3	Marked to complete inhibition	–	–

Escherichia coli
ATCC™ 25922



Formula

Difco™ Violet Red Bile Agar with MUG

Approximate Formula* Per Liter		
Yeast Extract	3.0	g
Peptone	7.0	g
Bile Salts No. 3	1.5	g
Lactose	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Neutral Red	0.03	g
Crystal Violet	2.0	mg
MUG (4-methylumbelliferyl-β-D-glucuronide)	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 41.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and use immediately.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Process each specimen as appropriate for that specimen.[1-3]
2. Incubate plates at 35°C for 22-26 hours.
3. Examine plates for growth and fluorescence.

Expected Results

Coliform organisms form purplish-red colonies that are generally surrounded by a reddish zone of precipitated bile. When examined under long-wave fluorescent light, MUG-positive colonies are surrounded by a bluish fluorescent halo. MUG-negative colonies lack the fluorescent halo.

E. coli colonies are red surrounded by a zone of precipitated bile and fluoresce blue under long-wave UV light.

Salmonella and *Shigella* strains that produce glucuronidase may be encountered infrequently but these are generally lactose negative and appear as colorless colonies which may fluoresce.

Limitations of the Procedure

1. Glucuronidase-negative strains of *E. coli* have been encountered.[5-7] Similarly, glucuronidase-positive strains of *E. coli* that do not fluoresce have been reported.[8]
2. Strains of *Salmonella* and *Shigella* that produce glucuronidase may infrequently be encountered.[9] These strains must be distinguished from *E. coli* on the basis of other parameters; e. g., gas production, lactose fermentation or growth at 44.5°C.

References

1. Davidson, Roth, and Gambrel-Lenarz. 2004. *In* Wehr and Frank (ed.). Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
2. Kornacki and Johnson. 2001. *In* Downes and Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Feng and Hartman. 1982. Appl. Environ. Microbiol. *43*:1320.
5. Chang, Brill and Lum. 1989. Appl. Environ. Microbiol. *55*:335.
6. Hansen and Yourassowsky. 1984. J. Clinical Microbiol. *20*:1177.
7. Kilian and Bulow. 1976. Acta Pathol. Microbiol. Scand. Sect. B *84*:245.
8. Mates and Shaffer. 1989. J. Appl. Bacteriology *67*:343.
9. Damare, Campbell and Johnston. 1985. J. Food Sci. *50*:1736.

Availability

Difco™ Violet Red Bile Agar with MUG

BAM COMPF

Cat. No. 229100 Dehydrated –500 g

BBL™ Violet Red Bile Agar with MUG

BAM COMPF

Cat. No. 299128 Prepared Bottle (200 mL) – Pkg. of 10

U-Z

Violet Red Bile Glucose Agar

Intended Use

Violet Red Bile Glucose Agar is used for detecting and enumerating *Enterobacteriaceae* in food and dairy products.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

The *Enterobacteriaceae* group includes lactose-fermenting coliform bacteria, lactose-nonfermenting strains of *E. coli*, and lactose-nonfermenting species, such as *Salmonella* and *Shigella*. When examining some foods, it is desirable to detect *Enterobacteriaceae* rather than the coliform bacteria.[4]

Enterobacteriaceae are glucose-fermenting bacteria. Mossel et al.[5] modified lactose-containing Violet Red Bile Agar by adding glucose to improve the recovery of *Enterobacteriaceae*. Later work by Mossel et al.[6,7] demonstrated that lactose could be omitted, resulting in the formulation known as Violet Red Bile Glucose Agar (VRBGA).

Violet Red Bile Glucose Agar is recommended for the detection and enumeration of *Enterobacteriaceae* in food and dairy products.[8,9] Violet Red Bile Glucose Agar is also listed in the *USP* as the recommended solid medium for use in the isolation of bile-tolerant gram-negative bacteria from nonsterile pharmaceutical products.[1]

The Violet Red Bile Glucose Agar formulation is available as a dehydrated culture medium, as prepared plated media, and in **Difco™ Hycheck™** *Enterobacteriaceae* hygiene contact slides. **Hycheck™** *Enterobacteriaceae* slides are double-sided paddles containing both Violet Red Bile Glucose Agar and Tryptic Soy Agar surfaces for immersing into fluids or for sampling surfaces.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

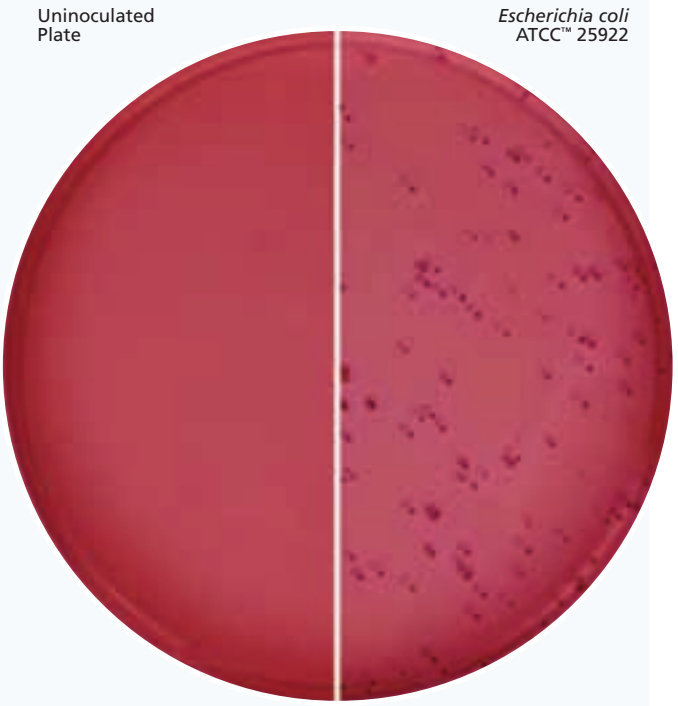
Identity Specifications

Difco™ Violet Red Bile Glucose Agar

Dehydrated Appearance:	Pink-beige to pink, free-flowing, homogeneous (may contain small dark particles).
Solution:	4.15% solution, soluble in purified water upon boiling. Solution is reddish purple, very slightly to slightly opalescent.
Prepared Appearance:	Reddish purple, very slightly to slightly opalescent.
Reaction of 4.15% Solution at 25°C:	pH 7.4 ± 0.2

BBL™ Violet Red Bile Glucose Agar (prepared)

Appearance:	Reddish to purple and opalescent.
Reaction at 25°C:	pH 7.4 ± 0.2



Cultural Response

Difco™ Violet Red Bile Glucose Agar

Prepare the medium per label directions. Using the pour plate method, inoculate and incubate at 35 ± 2°C for 18-24 hours. Using the streak plate method, inoculate and incubate (*) cultures at 30-35°C and (**) culture at 35-37°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Acinetobacter baumannii	19606	~10^3	None to poor	Colorless to red colonies, no bile ppt
Escherichia coli	25922	100-300	Good	Red to purple colonies, with bile ppt
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	100-300	Good	Red to purple colonies, with bile ppt
Staphylococcus aureus	25923	~10^3	None to poor	Colorless to red colonies, no bile ppt
*Escherichia coli**	8739	<100	Growth (30-35°C)	N/A
*Escherichia coli***	8739	<100	Growth (35-37°C)	N/A
*Pseudomonas aeruginosa**	9027	<100	Growth (30-35°C)	N/A

BBL™ Violet Red Bile Glucose Agar (prepared)

Inoculate plates and incubate *E. coli* strains and *P. aeruginosa* at 30-35°C for 18-24 hours. Incubate *S.* Typhimurium and *S. aureus* at 35-37°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Escherichia coli	25922	10-100	Good	Red to purple colonies, with bile ppt
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Red to purple colonies, with bile ppt
Staphylococcus aureus	6538	10^3-10^4	None to poor	Colorless to red colonies, no bile ppt
Escherichia coli	8739	10-100	Growth	N/A
Pseudomonas aeruginosa	9027	10-100	Growth	N/A

Principles of the Procedure

Violet Red Bile Glucose Agar contains pancreatic digest of gelatin as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Glucose is a carbohydrate. Bile salts and crystal violet inhibit gram-positive bacteria. Glucose fermenters produce red colonies with red-purple halos (bile precipitation) in the presence of neutral red, a pH indicator. Sodium chloride maintains the osmotic balance. Agar is the solidifying agent.

Formula

Difco™ Violet Red Bile Glucose Agar

Approximate Formula* Per Liter

Yeast Extract	3.0 g
Pancreatic Digest of Gelatin	7.0 g
Bile Salts No. 3	1.5 g
Glucose	10.0 g
Sodium Chloride	5.0 g
Neutral Red	0.03 g
Crystal Violet	2.0 mg
Agar	15.0 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 41.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for no more than 2 minutes to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[8,9]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For food samples, refer to appropriate standard references for details on test methods using Violet Red Bile Glucose Agar.[8,9]

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *Enterobacteriaceae* using Violet Red Bile Glucose Agar.[1]

This medium can be used in spread or pour plate procedures, with or without an overlay. In addition, this medium can be used as an overlayer for spread plates to both prevent swarming colonies and to provide semi-anaerobic conditions that suppress the growth of nonfermentative gram-negative organisms. Stab inoculation procedures can also be used with this medium.

Expected Results

Enterobacteriaceae ferment glucose, produce acid products and form red to dark purple colonies surrounded by red-purple halos.

Limitation of the Procedure

When used in the pour plate procedure, the medium should be freshly prepared, tempered to 47°C, and used within 3 hours.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Mossel. 1985. Int. J. Food Microbiol. 2:27.
5. Mossel, Mengerink and Scholts. 1962. J. Bacteriol. *84*:381.
6. Mossel, Eelderink, Koopmans and van Rossem. 1978. Lab Practice 27:1049.
7. Mossel, Eelderink, Koopmans and van Rossem. 1979. J. Food Protect. *42*:470.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
9. International Organization for Standardization. 2004. Microbiology of food and animal feeding stuffs – horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony count method. ISO 21528-2, 1st ed., 2004-08-15. International Organization for Standardization, Geneva, Switzerland.

Availability

Difco™ Violet Red Bile Glucose Agar

CCAM COMPF EP ISO JP USP

Cat. No. 218661 Dehydrated – 500 g†

BBL™ Violet Red Bile Glucose Agar

CCAM COMPF EP ISO JP USP

United States and Canada

Cat. No. 215198 Prepared Plates – Pkg. of 20*†
215053 Prepared Plates – Ctn. of 100*†

Europe

Cat. No. 254486 Prepared Plates – Pkg. of 20*†
257042 Prepared Contact Plates – Pkg. of 33*

Difco™ Hycheck™ Enterobacteriaceae Hygiene Contact Slides

Cat. No. 290003 Violet Red Bile Glucose Agar//Tryptic Soy Agar – Box of 10 slides*

**Store at 2-8°C.*
†QC testing performed according to USP/EP/JP performance specifications.

U-Z

Vitamin B_{12} Assay Medium

Intended Use

Vitamin B_{12} Assay Medium is used for determining vitamin B_{12} concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Vitamin B_{12} Assay Medium is prepared according to the formula described by Capp, Hobbs and Fox.[1] This medium is used in the microbiological assay of vitamin B_{12} using *Lactobacillus delbrueckii* subsp. *lactis* (*Lactobacillus leichmannii*) ATCC™ 4797 or 7830.

Principles of the Procedure

Vitamin B_{12} Assay Medium is a vitamin B_{12}-free medium containing all other nutrients and vitamins essential for the cultivation of *L. delbrueckii* subsp. *lactis* ATCC 4797 or 7830. To obtain

a standard curve, USP Cyanocobalamin Reference is added in specified increasing concentrations providing a growth response that can be measured titrimetrically or turbidimetrically.

Formula

Difco™ Vitamin B_{12} Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Acetate	20.0	g
L-Cystine	0.2	g
DL-Tryptophan	0.2	g
Adenine	20.0	mg
Guanine	20.0	mg
Uracil	20.0	mg
Xanthine	1.0	mg
Thiamine Hydrochloride	2.0	mg
Riboflavin	2.0	mg
Niacin	2.0	mg
Calcium Pantothenate	200.0	µg
Pyridoxine Hydrochloride	4.0	mg
p-Aminobenzoic Acid	200.0	µg
Biotin	10.0	µg
Folic Acid	100.0	µg
Polysorbate 80	2.0	g
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

User Quality Control

Identity Specifications

Difco™ Vitamin B_{12} Assay Medium

Dehydrated Appearance:	Very light to light beige, homogeneous, with a tendency to clump.
Solution:	3.8% solution (single-strength), soluble in purified water upon boiling 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear, may have a very slight precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Difco™ Vitamin B_{12} Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus delbrueckii* subsp. *lactis* ATCC™ 4797 when prepared single strength and supplemented with cyanocobalamin (vitamin B_{12}). The medium should produce a standard curve using a cyanocobalamin reference standard at 0.0 to 0.25 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Directions for Preparation from Dehydrated Product

1. Suspend 7.6 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL.
6. Autoclave at 121°C for 5 minutes.

Procedure

Stock cultures of the test organism, *L. delbrueckii* subsp. *lactis* ATCC™ 4797 or 7830, are prepared by stab inoculation of Lactobacilli Agar AOAC or B_{12} Culture Agar. Following incubation at 37°C for 24-48 hours, the tubes are stored in the refrigerator. Transfers are made at 2 week intervals.

Inoculum for the assay is prepared by subculturing a stock of *L. delbrueckii* subsp. *lactis* ATCC 4797 or 7830 into a tube containing 10 mL of Lactobacilli Broth AOAC or B_{12} Inoculum Broth. After incubation at 35-37°C for 18-24 hours, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are washed by resuspending in 10 mL of sterile 0.85% saline solution and centrifuging. The washing is repeated for a total of 3 times. Finally the cells are resuspended in 10 mL of sterile 0.85% saline. The cell suspension is then diluted 1:100 with sterile 0.85% saline. One drop is used to inoculate each assay tube.

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation that influence the standard curve readings cannot always be duplicated.

The concentrations required for the preparation of the standard curve are obtained by adding sufficient 25% ethanol to an accurately weighed amount of USP Cyanocobalamin Reference Standard (resulting in a solution containing 1.0 µg of cyanocobalamin per mL). This stock solution is stored in the refrigerator and should be used within 60 days. In the preparation of the standard curve, further dilutions of this stock solution (1 µg/mL) are made as follows:

A. Add 1 mL stock solution to 99 mL purified water (1 mL = 10 ng).
B. Add 1 mL of the solution from step A to 199 mL purified water (1 mL = 0.05 ng).

An acceptable standard curve can be obtained by using the USP Cyanocobalamin Reference Standard at levels of 0.0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 ng per assay tube. This is accomplished by adding 0, 0.5, 1, 2, 3, 4 and 5 mL of the 0.05 ng/mL solution per assay tube and sufficient purified water to make 10 mL volume per tube.

A standard concentration is used which, after incubation, gives a transmittance value at the 5 mL level of not less than that which corresponds to a dry cell weight of 1.25 mg (see *USP*[2] for method of calibration of a spectrophotometer and determination of dry cell weight). For the titrimetric method, a standard concentration should be used which, after incubation, will give a titration at the 5 mL level of 8-12 mL 0.1N sodium hydroxide.

Inoculate and incubate at 35-37°C for 18-24 hours. For turbidimetric determinations, place tubes in a refrigerator at 2-8°C for 15-20 minutes to stop growth. The growth can be measured by a nephelometric method. Titrimetric determinations of growth are made after incubation at 37°C for 72 hours. The curve is then constructed from the values obtained.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. Capps, Hobbs and Fox. 1949. J. Biol. Chem. *178*:517.
2. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Vitamin B_{12} Assay Medium

Cat. No. 236010 Dehydrated – 100 g*

**Store at 2-8°C.*

Vitamin K_1 - Hemin Solution

Intended Use

Vitamin K_1 - Hemin Solution is used as a culture medium enrichment for anaerobic microorganisms.

Summary and Explanation

CDC Anaerobe 5% Blood Agar was developed at the Centers for Disease Control and Prevention as a nonselective medium for the isolation and cultivation of a wide variety of obligately anaerobic microorganisms, particularly those found in clinical materials.[1,2] The medium is enriched with vitamin K_1 and hemin.

Principles of the Procedure

Gibbons and MacDonald reported isolating strains of *Bacteroides melaninogenicus* (*Prevotella melaninogenica*) that require hemin and vitamin K_1 for growth.[3] Vitamin K_1 enhances the growth of some strains of *Bacteroides* and certain gram-positive nonsporeformers.[4] The inclusion of Vitamin K_1 - Hemin in anaerobic culture media has been suggested by many investigators.[5]

Formula

BBL™ Vitamin K_1-Hemin Solution

Approximate Formula* Per Liter		
Hemin	0.5	g
Sodium Hydroxide	0.4	g
Phytomenadione (Vitamin K_1)	0.05	g
Ethyl Alcohol, 95%	10.0	mL

**Adjusted and/or supplemented as required to meet performance criteria.*

Procedure

Vitamin K_1-Hemin Solution is a ready-to-use solution. The solution cannot be heated and must be added aseptically in the proper amounts to media that have been autoclaved and cooled to 50-55°C.

Vitamin K_1-Hemin Solution is usually employed in prepared media at a final concentration of 5% for optimal results. Add Vitamin K_1-Hemin Solution as required.

Expected Results

Refer to appropriate references and procedures for results.

User Quality Control

Identity Specifications

BBL™ Vitamin K_1-Hemin Solution

Appearance: Dark blackish-brown, opaque.

Cultural Response

BBL™ Vitamin K_1-Hemin Solution

Prepare medium with added Vitamin K_1-Hemin Solution. Inoculate and incubate at 35-37°C for up to 4 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides levii	29147	10^3-10^4	Good
Bacteroides vulgatus	8482	10^3-10^4	Good

References

1. Dowell, Lombard, Thompson and Armfield. 1977. Media for isolation, characterization and identification of obligately anaerobic bacteria. CDC laboratory manual. CDC, Atlanta, Ga.
2. Jousimies-Somer, Summanen and Finegold. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Gibbons and MacDonald. 1960. J. Bacteriol. *80*:164.
4. Finegold, Sutter, Attebery and Rosenblatt. 1974. *In* Lennette, Spaulding and Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
5. Murray and Citron. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Vitamin K_1 - Hemin Solution

Cat. No. 212354 Prepared Tubes, 10 mL – Pkg. of 10*

**Store at 2-8°C.*

Vogel and Johnson Agar

(See VJ Agar)

WL Nutrient Medium • WL Nutrient Broth WL Differential Medium

Intended Use

WL Nutrient Medium and WL Nutrient Broth are used for cultivating yeasts, molds and bacteria encountered in brewing and industrial fermentation processes.

WL Differential Medium is used for isolating bacteria encountered in brewing and industrial fermentation processes.

Summary and Explanation

WL (Wallerstein Laboratory) nutrient media were developed by Green and Gray[1,2] in their study of various fermentation processes. An exhaustive study examining the methods of fermentation control procedures in worts, beers, liquid yeasts and similar fermentation products led to the development of these media.

At a pH of 5.5, counts of viable bakers' yeast may be made on the WL Nutrient Medium. By adjusting the pH to 6.5, the medium is suitable for obtaining counts of bakers' and distiller's yeast. The medium can support the growth of bacteria, but unless the number of yeast cells is small the bacteria may not be detected. Due to this limitation, Green and Gray developed WL Differential Medium that inhibits the growth of yeasts without inhibiting the growth of bacteria present in beers.

WL Nutrient Medium and WL Differential Medium are used simultaneously as a set of three plates. One plate is prepared from WL Nutrient Medium and two plates from WL Differential Medium.[3] The WL Nutrient Medium plate is incubated aerobically to obtain a total count of mainly yeast colonies. A differential agar plate is incubated aerobically for growth of acetic acid bacteria, *Flavobacterium*, *Proteus* and thermophilic bacteria. Another differential agar plate is incubated anaerobically for growth of lactic acid bacteria and *Pediococcus*.

Principles of the Procedure

Yeast extract is a source of trace elements, vitamins and amino acids. Peptone provides nitrogen, amino acids and carbon. Dextrose is the source of carbohydrate. Monopotassium phosphate buffers the media. Potassium chloride, calcium chloride and ferric chloride are essential ions and help to maintain osmotic balance. Magnesium sulfate and manganese sulfate are sources of divalent cations. Bromcresol green is a pH indicator. Agar is the solidifying agent in WL Nutrient Medium and WL Differential Medium. Cycloheximide inhibits yeasts and molds in WL Differential Medium.

Formulae

Difco™ WL Nutrient Medium

Approximate Formula* Per Liter		
Yeast Extract	4.0	g
Pancreatic Digest of Casein	5.0	g
Dextrose	50.0	g
Monopotassium Phosphate	0.55	g
Potassium Chloride	425.0	mg
Calcium Chloride	125.0	mg
Magnesium Sulfate	125.0	mg
Ferric Chloride	2.5	mg
Manganese Sulfate	2.5	mg
Agar	20.0	g
Bromcresol Green	22.0	mg

Difco™ WL Nutrient Broth

Consists of the same ingredients without the agar.

Difco™ WL Differential Medium

Consists of the same ingredients as WL Nutrient Medium with the addition of 4.0 mg cycloheximide.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ WL Nutrient Medium* – 80 g;
   Difco™ WL Differential Medium* – 80 g;
   Difco™ WL Nutrient Broth – 60 g.**
   Mix thoroughly.

   ** If desired, the pH may be adjusted to 6.5 ± 0.2 by adding approximately 27-32 mL (see label directions) of 1% sodium carbonate solution per liter of purified water prior to dissolving the powder.*

   *** If desired, add fermentation vials to tubes before autoclaving to assess gas production.*
2. Heat the agar media with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave agar and broth media at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ WL Nutrient Medium or WL Differential Medium

Dehydrated Appearance:	Light beige with a greenish tint, free-flowing, homogeneous.
Solution:	8.0% solution, soluble in purified water upon boiling. Solution is blue to greenish blue, very slightly to slightly opalescent.
Prepared Appearance:	Blue to greenish blue, slightly opalescent.
Reaction of 8.0% Solution at 25°C:	pH 5.5 ± 0.2

Difco™ WL Nutrient Broth

Dehydrated Appearance:	Light beige with a greenish tint, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in purified water. Solution is blue, clear.
Prepared Appearance:	Blue, clear.
Reaction of 6.0% Solution at 25°C:	pH 5.5 ± 0.2

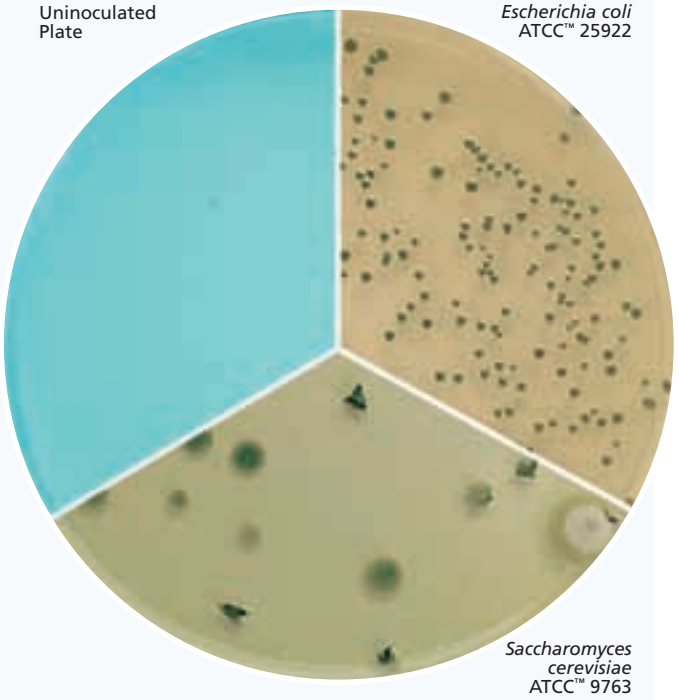
Uninoculated Tube | *Escherichia coli* ATCC™ 25922 | *Lactobacillus fermentum* ATCC™ 9338

Cultural Response

Difco™ WL Nutrient Medium

Prepare the medium per label directions. Using the pour plate technique, inoculate and incubate for 42-72 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Fair to good
Lactobacillus fermentum	9338	10^2-10^3	Fair to good
Saccharomyces cerevisiae	9763	10^2-10^3	Good



Uninoculated Plate | *Escherichia coli* ATCC™ 25922 | *Saccharomyces cerevisiae* ATCC™ 9763

Difco™ WL Nutrient Broth

Prepare the medium per label directions with the addition of inverted fermentation tubes for gas production. Inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and up to 5 days at 30 ± 2°C for yeasts.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID	GAS
Escherichia coli	25922	10^2-10^3	Fair to good	+	+
Lactobacillus fermentum	9338	10^2-10^3	Fair to good	+	sl. +
Saccharomyces cerevisiae	9763	10^2-10^3	Good	+	+

Acid + = positive, yellow
Acid – = negative, no color change

Difco™ WL Differential Medium

Prepare the medium per label directions. Using the pour plate technique, inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Lactobacillus fermentum	9338	5×10^2-10^3	Good
Saccharomyces cerevisiae	9763	10^3-2×10^3	Inhibition

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Green and Gray. 1950. Wallerstein Lab. Commun. *12*:43.
2. Green and Gray. 1950. Wallerstein Lab. Commun. *13*:357.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ WL Nutrient Medium

Cat. No. 242420 Dehydrated – 500 g

Difco™ WL Nutrient Broth

Cat. No. 247110 Dehydrated – 500 g

Difco™ WL Differential Medium

Cat. No. 242510 Dehydrated – 500 g

Wallenstein Medium

Intended Use
Wallenstein Medium is used in qualitative procedures for the isolation and cultivation of mycobacteria from clinical specimens.

Summary and Explanation
Wallenstein Medium is a glycerolated egg yolk medium containing malachite green.[1,2] Somewhat more inhibitory than Lowenstein-Jensen Medium because of the higher malachite green content, this medium is particularly recommended for use in parallel with other media for isolation of tubercle bacilli.[3]

Principles of the Procedure
Egg yolk is a source of energy and enhances the growth of *Mycobacterium* species. Inhibition of bacteria is achieved by the presence of malachite green dye.

Precaution
Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards.[4,5]

Procedure
The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.[5] N-acetyl-L-cysteine, sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. These reagents are provided in the **BBL™ MycoPrep™** Mycobacterial Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate reference.[3,5-7]

Following inoculation, keep test containers shielded from light and place in a suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ± 2°C.

Slanted and bottled media should be incubated in a horizontal plane until the inoculum is absorbed. Tubes and bottles should have screw caps loose for the first 3 weeks to permit circulation of carbon dioxide for the initiation of growth. Thereafter, to prevent dehydration, tighten caps; loosen briefly once a week. Stand tubes upright if space is a problem.

NOTE: Cultures from skin lesions suspected to be *M. marinum* or *M. ulcerans* should be incubated at 25-33°C for primary isolation. Incubate a duplicate culture at 35-37°C.[5]

Expected Results
Cultures should be read within 5-7 days after incubation and once a week thereafter for up to 8 weeks.

Record Observations[5]

1. Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7 days. Slow growers require more than 7 days for mature colony forms.
2. Number of colonies (bottles):
   No colonies = Negative
   Less than 50 colonies = Actual Count
   50 to 100 colonies = 1+
   100 to 200 colonies = 2+
   Almost confluent (200 to 500) = 3+
3. Pigment production
   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.

Bottles may be examined by inverting the bottles on the stage of a dissecting microscope. Read at 10-60× with transmitted light. Scan rapidly at 10-20× for the presence of colonies. Higher magnification (30-60×) is helpful in observing colony morphology; i.e., serpentine cord-like colonies.

References
1. Wallenstein. 1941. Am. J. Clin. Pathol., Tech. Sect. 11, 108.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
4. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
5. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. CDC, Atlanta, Ga.
6. Cernoch, Ennis, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.
7. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability
BBL™ Wallenstein Medium

Cat. No. 295857 Prepared Bottles – Ctn. of 100*

**Store at 2-8°C.*

Water

Intended Use
Water may be used in laboratory procedures; e.g., preparation of dilutions of reagents and suspensions of microorganisms.

Summary and Explanation
Water has been recommended for numerous uses in the microbiology laboratory.[1] It is utilized in serial dilutions for test cultures,

in preparing stock solutions of reagents and in the preparation of the base medium for yeast assimilation studies.[2]

Principles of the Procedure

The water in these tubes is purified (deionized) water that is ready and convenient for use as a diluent or suspending medium.

Reagent

Purified water.

References

1. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
2. Wickerham. 1951. Taxonomy of yeasts. U. S. Department of Agriculture technical bulletin 1029. Department of Agriculture, Washington, D.C.

Availability

BBL™ Water

Cat. No. 297345 Prepared Tubes (K Tubes), 5 mL – Ctn. of 100

Wilkins-Chalgren Agar • Anaerobe Broth MIC

Intended Use

Wilkins-Chalgren Agar is used for susceptibility testing of anaerobes and for isolating and cultivating anaerobes.

Anaerobe Broth MIC is used for susceptibility testing of anaerobes by the broth dilution technique.

Summary and Explanation

Wilkins-Chalgren Agar was designed by Wilkins and Chalgren[1] for use in determining the minimal inhibitory concentration (MIC) of antibiotics for anaerobic bacteria by the agar dilution procedure. This medium was recommended in the protocol used in the CLSI Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria.[2] More recently, Wilkins-Chalgren Agar has been replaced by Brucella Agar supplemented with laked sheep blood, hemin and vitamin K_1 as the recommended reference medium.[3]

Anaerobe Broth MIC is a modification of the formula described by Wilkins and Chalgren.[1] In Anaerobe Broth MIC the agar has been omitted.

The preferred medium for agar dilution tests with anaerobes is Wilkins-Chalgren Agar or Brucella Agar.[1] For broth microdilution tests Anaerobe Broth MIC has been used successfully.[4] Supplements must be added to these media to support the growth of certain fastidious anaerobes, including *Bacteroides gracilis*, *Bilophila wadsworthia*, *Prevotella* species, *Fusobacterium* species and anaerobic cocci.[4] Defibrinated sheep blood, 5% or lysed sheep blood is an adequate supplement for many fastidious anaerobic organisms.[2]

Principles of the Procedure

Peptones provide the nitrogen and amino acids in Wilkins-Chalgren Agar and Anaerobe Broth MIC. Yeast extract is the vitamin source in the media formulations. Dextrose is the carbon source, and sodium chloride maintains the osmotic balance of the media. L-arginine and sodium pyruvate are added to provide the proper environment for anaerobic growth. Hemin and vitamin K_1 are growth factors. Agar is the solidifying agent in Wilkins-Chalgren Agar.

User Quality Control

Identity Specifications

Difco™ Wilkins-Chalgren Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.8% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.8% Solution at 25°C:	pH 7.1 ± 0.1

Difco™ Anaerobe Broth MIC

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in purified water upon warming. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Light amber, clear, may have a slight precipitate.
Reaction of 3.3% Solution at 25°C:	pH 7.1 ± 0.1

Cultural Response

Difco™ Wilkins-Chalgren Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under anaerobic conditions for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
*Bacteroides fragilis**	25285	10^2-10^3	Good
*Bacteroides thetaiotaomicron**	29741	10^2-10^3	Good
Clostridium perfringens	13124	10^2-10^3	Good
Eubacterium lentum	43055	10^2-10^3	Good

Minimal Inhibitory Concentration (MIC) Assay: Prepare plates and inoculate as described by CLSI.[3] Test organisms marked (*) and compare the MIC (lowest concentration of antimicrobial that inhibits growth of the test bacterium) of the antimicrobials tested to the CLSI standard.[3]

Difco™ Anaerobe Broth MIC

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under anaerobic conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
*Bacteroides fragilis**	25285	10^2-3×10^2	Good
*Bacteroides thetaiotaomicron**	29741	10^2-3×10^2	Good
Eubacterium lentum	43055	10^2-3×10^2	Good

Minimal Inhibitory Concentration (MIC) Assay: Prepare broth microdilution trays and inoculate as described by CLSI.[3] Test organisms marked (*) and compare the MIC (lowest concentration of antimicrobial that inhibits growth of the test bacterium) of the antimicrobials tested to the CLSI standard.[3]

Formulae

Difco™ Wilkins-Chalgren Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Peptone	10.0	g
Yeast Extract	5.0	g
Dextrose	1.0	g
Sodium Chloride	5.0	g
L-Arginine	1.0	g
Sodium Pyruvate	1.0	g
Hemin	5.0	mg
Vitamin K_1	0.5	mg
Agar	15.0	g

Difco™ Anaerobe Broth MIC

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Wilkins-Chalgren Agar – 48 g;
   Difco™ Anaerobe Broth MIC – 33 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on susceptibility testing of anaerobic bacteria refer to appropriate procedures outlined in the references.[2-5]

Expected Results

Refer to appropriate references for acceptable ranges.

Limitation of the Procedure

Anaerobe Broth MIC is supplemented to a final concentration of 0.5 µg per mL of vitamin K_1 and 5.0 µg of hemin per mL. CLSI changed their recommendations to include use of broth with a final concentration of 1 µg of vitamin K_1 per mL.[2] To follow CLSI recommendations, the concentration of vitamin K_1 should be increased accordingly. A final concentration of 0.5 µg of vitamin K_1 per mL is sufficient, but some fastidious anaerobes may need a higher concentration of vitamin K_1.[5]

References

1. Wilkins and Chalgren. 1976. Antimicrob. Agents Chemother. *10*:926.
2. Clinical and Laboratory Standards Institute. 1993. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A3. CLSI, Wayne, Pa.
3. Clinical and Laboratory Standards Institute. 2001. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A5. CLSI, Wayne, Pa.
4. Wexler and Doern. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. Isenberg (ed.). 1995. Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Wilkins-Chalgren Agar

CCAM

Cat. No. 218051 Dehydrated – 500 g

Difco™ Anaerobe Broth MIC

CCAM

Cat. No. 218151 Dehydrated – 500 g

XL Agar Base • XLD Agar

Intended Use

XL (Xylose Lysine) Agar Base is used for the isolation and differentiation of enteric pathogens and, when supplemented with appropriate additives, as a base for selective enteric media.

XLD Agar is the complete Xylose Lysine Desoxycholate Agar, a moderately selective medium recommended for isolation and differentiation of enteric pathogens, especially *Shigella* species.

XLD Agar meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

A wide variety of media have been developed to aid in the selective isolation and differentiation of enteric pathogens. Due to the large numbers of different microbial species and strains with varying nutritional requirements and chemical resistance patterns, investigators have developed various formulae to meet general as well as specific needs relative to isolation and identification of the microorganisms.

XL Agar Base was developed by Taylor[4] for the nonselective isolation and differentiation of gram-negative enteric bacilli. It is particularly recommended for obtaining counts of enteric organisms. This medium can be rendered moderately selective for enteric pathogens, particularly *Shigella*, by the addition of sodium desoxycholate (2.5 g/L) to make XLD Agar.[4]

XL Agar Base can be made selective for *Salmonella* by adding 1.25 mL/L of 1% aqueous brilliant green to the base prior to autoclaving. Its use is recommended for *Salmonella* isolation after selenite or tetrathionate enrichment in food analysis; both coliforms and *Shigella* are inhibited.[4]

XLD Agar was developed by Taylor in order to increase the efficiency of the isolation and identification of enteric pathogens, particularly *Shigella*.[4] The pathogens are differentiated not only from the nonpathogenic lactose fermenters but also from many nonpathogens which do not ferment lactose or sucrose. Additionally, the medium was formulated to increase the frequency of growth of the more fastidious pathogens,[4] which in other formulations have often failed to grow due to the inclusion of excessively toxic inhibitors. The results obtained in a number of

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ XLD Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in purified water upon boiling. Solution is red, very slightly to slightly opalescent.
Prepared Appearance:	Red, slightly opalescent.
Reaction of 5.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ XLD Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. Incubate (*) cultures at 30-35°C for 18-48 hours and (**) culture at 35-37°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	~10^3	Partial inhibition	–
Escherichia coli	25922	~10^3	Partial inhibition	Yellow
Providencia alcalifaciens	9886	100-300	Good	Red
Shigella flexneri	12022	100-300	Good	Red
*Escherichia coli**	8739	>100	Partial to complete inhibition (30-35°C)	Yellow
Salmonella enterica subsp. *enterica* serotype Typhimurium*	14028	<100	Growth (30-35°C)	Red with black centers
Salmonella enterica subsp. *enterica* serotype Typhimurium**	14028	<100	Growth (35-37°C)	Red with black centers

Identity Specifications

BBL™ XL Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is dark medium to dark, red to rose-red, clear to slightly hazy.
Prepared Appearance:	Dark medium to dark, red to rose-red, clear to slightly hazy.
Reaction of 4.5% Solution at 25°C:	pH 7.5 ± 0.2

BBL™ XLD Agar (prepared)

Appearance:	Medium orange red to red, trace hazy.
Reaction at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ XL Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (up to 48 hours if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	10^3-10^4	Good	Yellow
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Red to yellow with black centers to predominantly black
Shigella flexneri	12022	10^3-10^4	Good	Red

BBL™ XLD Agar (prepared)

Inoculate and incubate at 35 ± 2°C for 24 hours. Incubate (*) cultures at 30-35°C for 18-48 hours and (**) culture at 35-37°C for 18-48 hours.

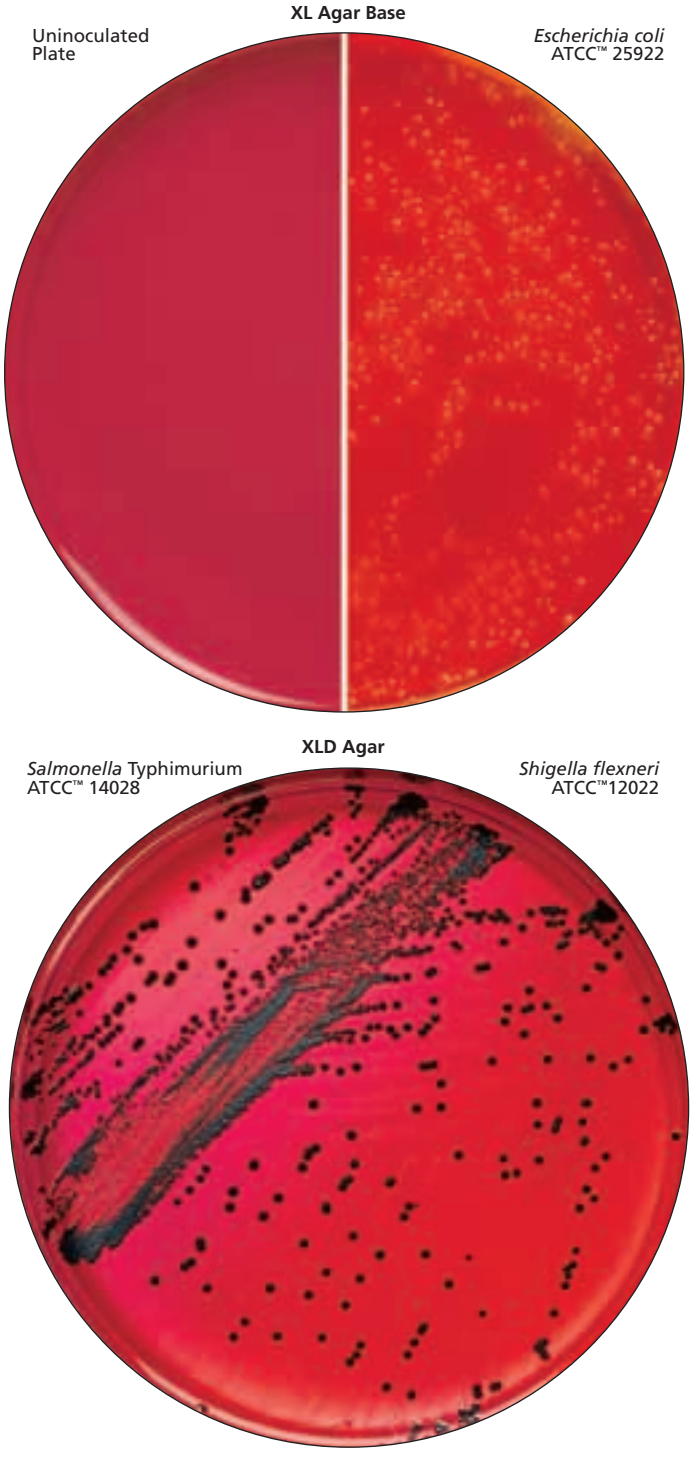
ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^4- 10^5	Partial inhibition	–
Escherichia coli	25922	10^4- 10^5	Partial to complete inhibition	Yellow to yellow-red
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Red with black centers
Shigella flexneri	12022	10^3-10^4	Good	Red
*Escherichia coli**	8739	10^2-10^3	Partial to complete inhibition (30-35°C)	Yellow to red
Salmonella enterica subsp. *enterica* serotype Typhimurium*	14028	<100	Growth (30-35°C)	Red with black centers
Salmonella enterica subsp. *enterica* serotype Typhimurium**	14028	<100	Growth (35-37°C)	Red with black centers

clinical evaluations have supported the claim for the relatively high efficiency of XLD Agar in the primary isolation of *Shigella* and *Salmonella*.[5-9]

XLD Agar is a selective and differential medium used for the isolation and differentiation of enteric pathogens from clinical specimens.[10-12] The value of XLD Agar in the clinical laboratory is that the medium is more supportive of fastidious enteric organisms such as *Shigella*.[12] XLD Agar is also recommended for the testing of food, dairy products and water in various industrial standard test methods.[13-17] General Chapter <62> of the *USP* describes the test method for the isolation of *Salmonella* from nonsterile pharmaceutical products using XLD Agar as the solid culture medium.[1]

Principles of the Procedure

Xylose is incorporated into the medium because it is fermented by practically all enterics except for the shigellae. This property enables the differentiation of *Shigella* species. Lysine is included to enable the *Salmonella* group to be differentiated from the non-pathogens. Without lysine, salmonellae rapidly would ferment



the xylose and be indistinguishable from nonpathogenic species. After the salmonellae exhaust the supply of xylose, the lysine is attacked via the enzyme lysine decarboxylase, with reversion to an alkaline pH, which mimics the *Shigella* reaction. To prevent similar reversion by lysine-positive coliforms, lactose and sucrose (saccharose) are added to produce acid in excess.[4] Degradation of xylose, lactose and sucrose generates acid products, which in the presence of the pH indicator phenol red, causes a color change in the medium from red to yellow.

To add to the differentiating ability of the formulation, an H_2S indicator system, consisting of sodium thiosulfate and ferric ammonium citrate, is included for the visualization of the hydrogen sulfide produced, resulting in the formation of colonies with black centers. The nonpathogenic H_2S producers do not decarboxylate lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies.[4] Sodium chloride maintains the osmotic balance. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Agar is the solidifying agent.

XLD Agar is both a selective and differential medium. It utilizes sodium desoxycholate as the selective agent and, therefore, it is inhibitory to gram-positive microorganisms.

Formulae

BBL™ XL Agar Base

Approximate Formula* Per Liter

Xylose	3.5	g
L-Lysine	5.0	g
Lactose	7.5	g
Sucrose	7.5	g
Sodium Chloride	5.0	g
Yeast Extract	3.0	g
Phenol Red	0.08	g
Agar	13.5	g

Difco™ XLD Agar

Approximate Formula* Per Liter

Xylose	3.5	g
L-Lysine	5.0	g
Lactose	7.5	g
Saccharose	7.5	g
Sodium Chloride	5.0	g
Yeast Extract	3.0	g
Phenol Red	0.08	g
Sodium Desoxycholate	2.5	g
Ferric Ammonium Citrate	0.8	g
Sodium Thiosulfate	6.8	g
Agar	13.5	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

BBL™ XL Agar Base

1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Add brilliant green, if desired.
3. Autoclave at 118°C for 10 minutes. Cool to 55-60°C.
4. Add 20 mL of an aqueous solution containing 34% sodium thiosulfate and 4% ferric ammonium citrate. For XLD agar, add 25 mL of 10% aqueous sodium desoxycholate. Pour into plates.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ XLD Agar

1. Suspend 55 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with agitation just until the medium boils. DO NOT OVERHEAT. DO NOT AUTOCLAVE.

3. Cool to 45-50°C in a water bath and use immediately. Overheating causes precipitation.
4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[10-12]

For food, dairy or water samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[13-17]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using XLD Agar.[10-12]

For food, dairy and water samples, refer to appropriate standard references for details on test methods using XLD Agar.[13-17]

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and the isolation of *Salmonella* using XLD Agar.[1]

A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. Colonies on XLD agar may require 48 hours incubation for full color development.

Expected Results

Degradation of xylose, lactose and sucrose generates acid products, causing a color change in the medium from red to yellow.

Hydrogen sulfide production under alkaline conditions causes colonies to develop black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.

Lysine decarboxylation in the absence of lactose and sucrose fermentation causes reversion to an alkaline condition and the color of the medium changes back to red.

Typical colonial morphology and reactions on XLD Agar are as follows:

E.coli	Large, flat, yellow; some strains may be inhibited
Enterobacter / Klebsiella	Mucoid, yellow
Proteus	Red to yellow; most strains have black centers
Salmonella	Red-yellow with black centers
Shigella, Salmonella H_2S-negative	Red
Pseudomonas	Red
Gram-positive bacteria	No growth to slight growth

Limitations of the Procedure

1. Red, false-positive colonies may occur with some *Proteus* and *Pseudomonas* species.
2. Incubation in excess of 48 hours may lead to false-positive results.
3. *S.* Paratyphi A, *S.* Choleraesuis, *S. pullorum* and *S. gallinarum* may form red colonies without black centers, thus resembling *Shigella* species.
4. Some *Proteus* strains will give black-centered colonies on XLD Agar.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Taylor. 1965. Am. J. Clin. Pathol. *44*:471.
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7. Taylor and Schelhart. 1967. Am. J. Clin. Pathol. *48*:356.
8. Taylor and Schelhart. 1968. Appl. Microbiol. *16*:1387.
9. Pollock and Dahlgren. 1974. Appl. Microbiol. *27*:197.
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14. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
15. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
16. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
17. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.

Availability

BBL™ XL Agar Base

SMWW

Cat. No. 211836 Dehydrated – 500 g

Difco™ XLD Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP ISO JP MCM9 SMD SMWW USP

Cat. No. 278850 Dehydrated – 500 g†
278820 Dehydrated – 2 kg†
278830 Dehydrated – 10 kg†

BBL™ XLD Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP ISO JP MCM9 SMD SMWW USP

United States and Canada

Cat. No. 221192 Prepared Plates – Pkg. of 20*†
221284 Prepared Plates – Ctn. of 100*†

Europe

Cat. No. 254055 Prepared Plates – Pkg. of 20*
254090 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 252020 Prepared Plates – Pkg. of 20*
251159 Prepared Plates – Ctn. of 100*

BBL™ XLD Agar//Hektoen Enteric Agar

Cat. No. 295646 Prepared **I Plate**™ Dishes – Pkg. of 20*

*Store at 2-8°C.
†QC testing performed according to USP/EP/JP performance specifications.

XLT4 Agar Base • XLT4 Agar Supplement

Intended Use

XLT4 Agar Base is used with XLT4 Agar Supplement in isolating non-typhi *Salmonella*.

Summary and Explanation

Numerous media have been developed for isolating and differentiating enteric pathogens. The majority were designed to recover a broad spectrum of enteric pathogens.[1] Consequently, overgrowth of nuisance or contaminating organisms can be a major problem when recovery of a specific organism or species is desired. This is particularly true for *Salmonella* isolation media where overgrowth of *Proteus*, *Providencia* and *Pseudomonas* can dramatically interfere with the detection and isolation of *Salmonella*.

In 1990, Miller and Tate described a new medium, XLT4 Agar, for isolating *Salmonella*.[1] The authors established the selectivity of XLT4 Agar using pure cultures of a variety of enteric organisms. They also evaluated its sensitivity in detecting and isolating *Salmonella* using fecal-contaminated farm samples containing high numbers of competing bacteria. In follow-up studies, Miller[2,3] and Tate[4] reported that XLT4 Agar significantly improved the recovery of non-typhi *Salmonella* from chicken and farm environmental drag-swab samples.

Principles of the Procedure

XLT4 Agar Base contains peptone as a source of complex nitrogen compounds. Yeast extract is added as a source of vitamins and other cofactors. Differentiation of *Salmonella* from other organisms that also grow on this medium is based on fermentation of xylose, lactose and sucrose, decarboxylation of lysine and the production of hydrogen sulfide. Hydrogen sulfide production is detected by the addition of ferric ions. Sodium thiosulfate is added as a source of inorganic sulfur. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. Phenol red is added as an indicator of pH changes resulting from fermentation and decarboxylation reactions. XLT4 Agar Supplement is added to inhibit growth of non-*Salmonella* organisms.

Formulae

Difco™ XLT4 Agar Base

Approximate Formula* Per Liter

Proteose Peptone No. 3	1.6	g
Yeast Extract	3.0	g
L-Lysine	5.0	g
Xylose	3.75	g
Lactose	7.5	g
Saccharose	7.5	g
Ferric Ammonium Citrate	0.8	g
Sodium Thiosulfate	6.8	g
Sodium Chloride	5.0	g
Agar	18.0	g
Phenol Red	0.08	g

User Quality Control

Identity Specifications

Difco™ XLT4 Agar Base

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	5.9% solution, soluble upon boiling in purified water containing 4.6 mL/L of XLT4 Agar Supplement. Solution is red, slightly opalescent.
Prepared Appearance:	Reddish-orange, slightly opalescent.
Reaction of Final Medium at 25°C:	pH 7.4 ± 0.2

Difco™ XLT4 Agar Supplement

Appearance:	Colorless to slightly yellow, clear, slightly viscous solution.

Cultural Response

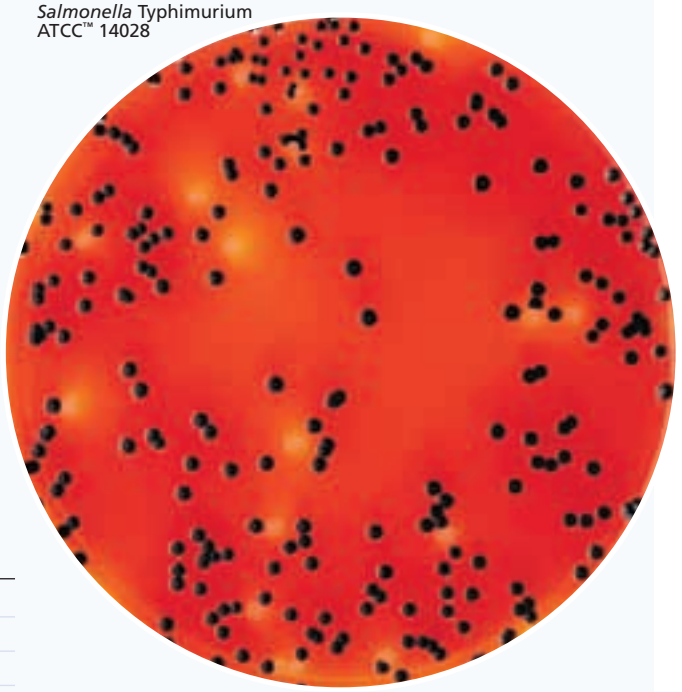
Difco™ XLT4 Agar Base with XLT4 Agar Supplement

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^3	Marked inhibition	–
Escherichia coli	25922	10^3	Partial inhibition	Yellow
Proteus mirabilis	25933	10^3	Inhibition	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Yellow to red with black centers
Staphylococcus aureus	25923	10^3	Inhibition	–

XLT4 Agar Base with XLT4 Supplement

Salmonella Typhimurium ATCC™ 14028



Difco™ XLT4 Agar Supplement

A 27% solution (approximate) of the surfactant Tergitol™** 4 (7-ethyl-2-methyl-4-undecanol hydrogen sulfate, sodium salt).

**Adjusted and/or supplemented as required to meet performance criteria.*
***Tergitol is a trademark of Union Carbide Chemicals & Plastics Technology Corporation.*

Directions for Preparation from Dehydrated Product

1. Suspend 59 g of the powder in 1 L of purified water.
2. Add 4.6 mL XLT4 Agar Supplement. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating. DO NOT AUTOCLAVE.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Inoculate a suitable *Salmonella* enrichment broth (such as Tetrithionate Broth) and incubate at 35°C for 18-24 hours.
2. Following enrichment, subculture onto XLT4 Agar. Streak for isolation.
3. Incubate plates aerobically at 35 ± 2°C. Examine for growth after 18-24 and 48 hours incubation.

Expected Results

Typical *Salmonella* colonies (H_2S-positive) appear black or black-centered with a yellow periphery after 18-24 hours of incubation. Upon continued incubation, the colonies become entirely black or pink to red with black centers.

Colonies of H_2S-negative *Salmonella* strains appear pinkish-yellow.

Most *Citrobacter* colonies that grow on this medium are yellow without evidence of blackening. Growth of *Enterobacter aerogenes* and *Escherichia coli* is markedly inhibited; colonies that do grow appear yellow without evidence of blackening. Growth of *Proteus*, *Pseudomonas*, *Providencia*, *Alteromonas putrefaciens*, *Yersinia enterocolitica* and *Acinetobacter calcoaceticus* is markedly to completely inhibited on XLT4 Agar. *Shigella* species are partially inhibited and colonies appear red.

Limitations of the Procedure

1. XLT4 Agar is intended for detecting and isolating *Salmonella* based on selectivity and colonial characteristics. Presumed *Salmonella* colonies must be confirmed by biochemical and/or immunological methods. Consult appropriate references for further information.[5-7]
2. Non-*Salmonella* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Salmonella*. Consult appropriate references.[5-7]
3. Freshly inoculated plates and plates held over several days may develop multicolored, metallic looking crystals/flecks on the surface. These crystals/flecks do not interfere with the performance of the medium.

References

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6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
7. Downes and Ito (ed.) 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ XLT4 Agar Base

USDA

Cat. No. 223420 Dehydrated – 500 g

Difco™ XLT4 Agar Supplement

USDA

Cat. No. 235310 Bottle – l00 mL

Xanthine Agar

(See Nocardia Differentiation Media)

Bacto™ Yeast Extract • Yeast Extract, UF Yeast Extract, LD • Bacto™ Yeast Extract, Technical Yeast Extract

Intended Use

Bacto Yeast Extract, Yeast Extract, UF (ultra-filtered), Yeast Extract, LD, **Bacto** Yeast Extract, Technical and Yeast Extract are used in preparing microbiological culture media.

Summary and Explanation

Bacto Yeast Extract, Yeast Extract, UF, Yeast Extract, LD, **Bacto** Yeast Extract, Technical and Yeast Extract are concentrates of the water-soluble portion of *Saccharomyces cerevisiae* cells that have been autolyzed. The autolysis is carefully controlled to preserve the naturally occurring B-complex vitamins. Yeast extract is considered a non-animal product and is used extensively for many non-animal formulations for bacterial, fungal, mammalian and insect cell culture.

Bacto Yeast Extract has been considered one of the most complete and versatile of the fermentation bionutrients avail-

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™/Bacto™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Yeast Extract

Dehydrated Appearance: Light to medium beige, to medium tan, free-flowing, homogeneous.

Solution: 1.0% and 2.0% solutions, soluble in purified water. 1.0% solution is light to medium amber, clear, may have a very slight precipitate. 2.0% solution is medium amber, clear, may have a very slight precipitate.

Difco™ Yeast Extract, UF

Dehydrated Appearance: Fine, homogeneous, may contain up to a small amount of minute light to dark tan particles.

Solution: 2.0% solution, soluble in purified water. Solution is light to dark, yellow to tan, clear to slightly hazy.

Reaction of 2.0% Solution at 25°C: pH 6.3-7.3

Difco™ Yeast Extract, LD

Dehydrated Appearance: Fine, homogeneous, may contain up to a small amount of minute light to dark tan particles.

Solution: 2.0% solution, soluble in purified water. Solution is light to dark, yellow to tan, clear to slightly hazy.

Bacto™ Yeast Extract, Technical

Dehydrated Appearance: Light to medium beige, free-flowing, homogeneous.

Solution: 2.0% solution, soluble in purified water. Solution is medium amber, clear to very slightly opalescent.

Cultural Response

Bacto™ Yeast Extract

Prepare a solution containing 1% **Bacto** Yeast Extract and 0.5% sodium chloride. Adjust the pH to 7.2 ± 0.2 using dilute NaOH. Dispense into tubes and autoclave. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	30-300	Fair to good
Staphylococcus aureus	25923	30-300	Good
Streptococcus pneumoniae	6305	30-300	Good

Bacto™ Yeast Extract, Technical

Prepare a solution containing 2% **Bacto** Yeast Extract, Technical and 0.5% sodium chloride. Adjust the pH to 7.2-7.4 using dilute NaOH. Dispense into tubes and autoclave. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	30-300	Good
Streptococcus pyogenes	19615	30-300	Good

Identity Specifications

BBL™ Yeast Extract

Dehydrated Appearance: Fine, homogeneous, may contain up to a small amount of minute light to dark, tan particles.

Solution: 2.0% solution, soluble in purified water. Solution is clear to slightly hazy.

Reaction of 2.0% Solution at 25°C: pH 5.4-7.2

Cultural Response

BBL™ Yeast Extract

Prepare a sterile solution containing 10.0 g of Yeast Extract, 2.5 g of sodium chloride and 6.5 g of agar in 500 mL of purified water. Adjust the final pH to 7.2-7.5. Inoculate and incubate plates at 35 ± 2°C for 3 days (incubate streptococci with 3-5% CO_2; incubate *C. sporogenes* anaerobically).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Candida albicans	10231	10^3-10^4	Good
Clostridium sporogenes	11437	10^3-10^4	Good
Escherichia coli	25922	10^3-10^4	Good
Kocuria rhizophila	9341	10^3-10^4	Good
Streptococcus pneumoniae	6305	10^3-10^4	Good
Streptococcus pyogenes	49117	10^4-10^5	Good

able. It has been a valuable ingredient for the microbiological assay of vitamins. Yeast extract is also of value in the assay of antibiotics. B factor, a growth substance necessary for the production of rifampin in a *Nocardia* sp., can be isolated from yeast extract.[1]

Yeast Extract, UF is ultra-filtered and specifically designed for tissue culture applications. With its low endotoxin level and high content of naturally occurring B vitamins, it is an ideal substitute for fetal bovine serum. It has an endotoxin level of less than or equal to 500 EU/g.

Yeast Extract, LD was created to eliminate the problem of dust inhalation when handling large quantities of yeast extract. Yeast Extract, Yeast Extract, UF and Yeast Extract, LD are processed from the same culture of *Saccharomyces*.

Bacto Yeast Extract, Technical and Yeast Extract were developed to provide products priced for the biotechnology/pharmaceutical market with acceptable clarity and growth promoting characteristics.

Media formulations containing yeast extract are specified in standard methods for various applications.[2-8]

Principles of the Procedure

Bacto Yeast Extract, Yeast Extract, UF, Yeast Extract, LD, **Bacto** Yeast Extract, Technical and Yeast Extract are prepared by growing baker's yeast, *Saccharomyces* sp., in a carbohydrate-rich plant medium. The yeast is harvested, washed and resuspended

in water, where it undergoes autolysis, or self-digestion. Yeast extract is the total soluble portion of this autolytic action. The autolytic activity is stopped by a heating step. The resulting yeast extract is then filtered to produce a clear product and subsequently made into a powder by a spray-drying process.

Bacto Yeast Extract, Yeast Extract, UF, Yeast Extract, LD, **Bacto** Yeast Extract, Technical and Yeast Extract provide vitamins, nitrogen, amino acids and carbon in microbiological culture media.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of **Bacto** Yeast Extract, Yeast Extract, UF, Yeast Extract, LD, **Bacto** Yeast Extract, Technical and Yeast Extract in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for specific procedures using **Bacto** Yeast Extract, Yeast Extract, UF, Yeast Extract, LD, **Bacto** Yeast Extract, Technical and Yeast Extract.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Kawaguchi, Asahi, Satoh, Uozumi and Beppu. 1984. J. Antibiot. *37*:1587.
2. Horowitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. U.S. Environmental Protection Agency (USEPA). 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, Washington, D.C.
6. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
7. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
8. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Bacto™ Yeast Extract

AOAC | BAM | COMPF | EPA | SMD | SMWW | USDA

Cat. No.	212750	Dehydrated – 500 g
	212720	Dehydrated – 2 kg
	212730	Dehydrated – 10 kg

Difco™ Yeast Extract, UF

Cat. No.	210929	Dehydrated – 500 g
	210934	Dehydrated – 10 kg

Difco™ Yeast Extract, LD

Cat. No.	210933	Dehydrated – 500 g
	210941	Dehydrated – 10 kg

Bacto™ Yeast Extract, Technical

Cat. No.	288620	Dehydrated – 500 g
	288610	Dehydrated – 10 kg

BBL™ Yeast Extract

Cat. No.	211929	Dehydrated – 454 g
	211930	Dehydrated – 5 lb (2.3 kg)
	211931	Dehydrated – 25 lb (11.3 kg)

Yeast Extract Glucose Chloramphenicol Agar

Intended Use

Yeast Extract Glucose Chloramphenicol Agar is a selective agar recommended by the International Dairy Federation[1,2] for enumerating yeasts and molds in milk and milk products.

Summary and Explanation

The antibiotic method for enumerating yeasts and molds in dairy products has become the method of choice, replacing the traditional acidified method.[2] The use of antibiotics for suppressing bacteria results in better recovery of injured fungal cells, which are sensitive to an acid environment, and in less interference from precipitated food particles during the counting.[3-7]

Yeast Extract Glucose Chloramphenicol Agar is a nutrient medium that inhibits the growth of organisms other than yeasts and molds due to the presence of chloramphenicol. When a sample contains predominantly yeasts and/or injured yeasts, the use of Yeast Extract Glucose Chloramphenicol Agar may offer some advantage.[2] After incubation at 25°C, colonies are counted and yeast colonies are distinguished from molds by colony morphology.

Principles of the Procedure

Yeast extract provides basic nutrients. Glucose is a carbon energy source. Chloramphenicol inhibits bacterial growth. Agar is the solidifying agent.

Formula

Difco™ Yeast Extract Glucose Chloramphenicol Agar

Approximate Formula* Per Liter		
Yeast Extract	5.0	g
Glucose	20.0	g
Chloramphenicol	0.1	g
Agar	13.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 38.1 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Yeast Extract Glucose Chloramphenicol Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.81% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 3.81% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Difco™ Yeast Extract Glucose Chloramphenicol Agar

Prepare the medium per label directions. Inoculate by the pour plate technique and incubate at 25 ± 2°C for up to 4 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	30-300	Good
Candida albicans	10231	30-300	Good
Escherichia coli	25922	10^3-2×10^3	Inhibition
Saccharomyces cerevisiae	9763	30-300	Good

Procedure

1. Prepare initial sample dilutions using 10 g or 10 mL of sample in 90 mL of diluent, as listed below:

SAMPLE 10 g or 10 mL	DILUENT 90 mL	PREPARATION
Milk Liquid milk product	1/4-strength Ringer's solution	Mix.
Dried Milk Whey powder Buttermilk powder Lactose	1/4-strength Ringer's solution	Shake at 47°C.
Casein	2% dipotassium phosphate solution	Shake at 47°C.
Cheese	2% sodium citrate solution	Shake at 47°C.
Butter Edible ice	1/4-strength Ringer's solution	Shake at 47°C.
Custard dessert Fermented milk Yogurt	1/4-strength Ringer's solution	Shake.

2. Add 10 mL from the initial dilution prepared above (#1) to 90 mL of 1/4-strength Ringer's solution. One milliliter (1 mL) of this dilution corresponds to 0.01 g/mL of sample.
3. Prepare further dilutions by adding 10 mL of the 0.01 g/mL dilution above (#2) to 90 mL of diluent.
4. Pipette 1 mL of each dilution into two Petri dishes.
5. Pour 10 mL of sterile molten agar (cooled to 45°C) into each dish. Mix thoroughly.
6. Incubate at 25°C for 4 days.

Expected Results

1. Select plates containing 10-300 colonies and count the colonies. Distinguish yeasts from molds by colony morphology.
2. Express results as yeasts and molds "per gram" or "per milliliter."

References

1. International Dairy Federation. 2004. Standard Method ISO 6611/IDF 94.
2. Frank and Yousef. 2004. *In* Frank and Wehr (ed.), Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
3. Beuchat. 1979. J. Food Prot. *42*:427.
4. Cooke and Brazis. 1968. Mycopathol. Mycol. Appl. *35*:281.
5. Koburger. 1970. J. Milk Food Technol. *33*:433.
6. Koburger. 1973. J. Milk Food Technol. *36*:434.
7. Overcase and Weakley. 1969. J. Milk Food Technol. *32*:442.

Availability

Difco™ Yeast Extract Glucose Chloramphenicol Agar

ISO SMD

Cat. No. 219001 Dehydrated – 500 g

Yeast Extract-Peptone-Dextrose (YPD) Agar
Yeast Extract-Peptone-Dextrose (YPD) Broth

Intended Use

YPD Agar and YPD Broth are used for maintaining and propagating yeasts in molecular microbiology procedures.

Summary and Explanation

General methods in yeast genetics specify using yeast extract-peptone-dextrose (YPD) medium for cultivating *Saccharomyces cerevisiae* and other yeasts.[1] Yeasts grow well on a minimal medium containing only dextrose and salts. The addition of protein and yeast cell extract hydrolysates allows faster growth so that during exponential or log-phase growth, the cells divide every 90 minutes.[1]

Principles of the Procedure

YPD Agar and YPD Broth contain peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. YPD Agar contains agar as the solidifying agent.

Formulae

Difco™ YPD Agar

Approximate Formula* Per Liter

Yeast Extract	10.0	g
Peptone	20.0	g
Dextrose	20.0	g
Agar	15.0	g

Difco™ YPD Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ YPD Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 6.5 ± 0.2

Difco™ YPD Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

Difco™ YPD Agar or YPD Broth

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for 42-48 hours (broth) or 48 hours (agar – up to 72 hours if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Kluyveromyces lactis	8563	10^2-10^3	Good
Saccharomyces cerevisiae	18790	10^2-10^3	Good
Saccharomyces cerevisiae	9080	10^2-10^3	Good

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ YPD Agar – 65 g;
   Difco™ YPD Broth – 50 g.
   Mix thoroughly.
2. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave the agar and broth media at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Growth of colonies on the agar or turbidity in the broth.

Reference

1. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl. 1994. Current protocols in molecular biology, Current Protocols, Brooklyn, N.Y.

Availability

Difco™ YPD Agar

Cat. No.	242720	Dehydrated – 500 g
	242710	Dehydrated – 2 kg

Difco™ YPD Broth

Cat. No.	242820	Dehydrated – 500 g
	242810	Dehydrated – 2 kg

U-Z

Yeast Extract Phosphate (YEP) Agar

Intended Use

YEP Agar is used in qualitative procedures for the isolation of dimorphic pathogenic fungi from clinical specimens. The plates are deep-filled to reduce the effects of drying during prolonged incubation.

Summary and Explanation

Smith and Goodman developed YEP Agar for the primary recovery of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from contaminated specimens.[1] The medium is designed to be used with ammonium hydroxide, a selective agent that improves the recovery of dimorphic pathogens by inhibiting bacteria, yeasts and saprophytic fungi.[2,3]

Principles of the Procedure

Yeast extract primarily supplies B-complex vitamins, but also provides some amino acids and carbohydrates to support fungal growth. A phosphate buffer maintains a slightly acidic pH. Chloramphenicol is a broad spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria. A drop of ammonium hydroxide (NH_4OH) added to the surface of the inoculated medium inhibits bacteria, yeasts and saprophytic fungi that may be contained in clinical specimens without affecting the growth of *Blastomyces* or *Histoplasma* spp. Phenol red changes the color of the medium from pale tan to pink to show that the NH_4OH has been applied to the agar surface. It also shows the loss of alkalinity as

the ammonia volatilizes and the pH falls below 7.0. Since *Histoplasma* and *Blastomyces* spp. grow more slowly than bacteria or yeasts they are probably less affected by the transient high pH produced by NH_4OH.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Add one drop of concentrated NH_4OH (ammonia) at the edge of the inoculated medium and allow the medium to sit for 20 minutes before inverting.

Incubate the plates in an inverted position (agar side up) at 22-25°C.

Expected Results

All cultures should be examined for growth at least weekly. Cultures should be held for 4-6 weeks before reporting as negative.

References

1. Smith and Goodman. 1974. Am. J. Clin. Pathol. 62:276.
2. Haley and Callaway. 1978. Laboratory methods in medical mycology. HEW Publication No. (CDC) 78-8361. Center for Disease Control, Atlanta, Ga.
3. Larone, Mitchell and Walsh. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Yeast Extract Phosphate (YEP) Agar

Cat. No. 297886 Prepared Plates (Deep Fill) – Pkg. of 10*

**Store at 2-8°C.*

2× Yeast Extract Tryptone (2×YT) Medium

Intended Use

2×YT Medium is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

2×YT Medium is a nutritionally rich growth medium designed for growth of recombinant strains of *Escherichia coli*. This medium is also used for propagation of M13 bacteriophage for sequencing and phage display research.[1-3] The components of 2×YT Medium provide nitrogen and growth factors that allow bacteriophage to reproduce in large quantities without exhausting the host. *E. coli* grows more rapidly in this rich medium because it provides amino acids, nucleotide precursors, vitamins and other metabolites that the cell would otherwise have to synthesize.[2]

Principles of the Procedure

Peptone and yeast extract provide the necessary nutrients and cofactors required for excellent growth of *E. coli*. Sodium chloride is included to provide a suitable osmotic environment.

Formula

Difco™ 2×YT Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	16.0	g
Yeast Extract	10.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil when necessary for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ 2×YT Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.1% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ 2×YT Medium

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli (C600)	23724	10^2-3×10^2	Good

Procedure

Consult appropriate references for recommended test procedures.[1-3]

Expected Results

Growth is evident in the form of turbidity.

References

1. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl. 1994. Current protocols in molecular biology, vol 1. Current Protocols, New York, N.Y.
3. Davis, Dibner and Battey. 1986. Basic methods in molecular biology. Elsevier, New York, N.Y.

Availability

Difco™ 2×YT Medium

Cat. No. 244020 Dehydrated – 500 g
244010 Dehydrated – 2 kg

Yeast Fermentation Broth Base with Durham Tube
Yeast Fermentation Broth with Carbohydrates and Durham Tube

Intended Use
Yeast Fermentation Broth media are used for identification of yeasts based on the fermentation of specific carbohydrates; e.g., dextrose, galactose, lactose, maltose, sucrose, trehalose and xylose.[1] A Durham tube is provided to detect the gas produced during fermentation.

Summary and Explanation
Yeast Fermentation Broth is a modification of a medium developed by Wickerham for the determination of carbohydrate fermentation by yeasts.[2] In this test, tubes of media, each containing a specific carbohydrate, are inoculated with a yeast isolate. If the carbohydrate is fermented by the yeast, the color of the medium changes from purple to yellow, due to the formation of acids, and gas is produced.

In this modification of the Wickerham formula, bromcresol purple is substituted for bromthymol blue.

Principles of the Procedure
Yeast Fermentation Broth Base consists of a peptone medium supplemented with yeast extract to supply the B complex vitamins necessary to support growth. Specific carbohydrates are added to the basal medium in a concentration of 1%.

When the medium is inoculated with a yeast that is able to ferment the carbohydrate present, acids or acids and gas are produced. The acids lower the pH of the medium and change the indicator from purple to yellow. Gas production is the most reliable evidence of the fermentation of carbohydrates by yeasts because some yeasts can assimilate certain carbohydrates without fermenting them.[2] Gas production is detected by the displacement of the medium in the Durham tube, leaving a clear area (bubble) in the tube.

The pattern of fermentation of a battery of carbohydrates is characteristic of a given species or group of yeast species and may be used for their classification or identification.

Procedure
Subculture the isolate to be identified onto a Sabouraud Dextrose Agar slant or **Mycophil**™ Agar slant.

Air bubbles should be removed from the Durham tube prior to inoculation by inverting the broth tube and gently tapping the side to dislodge the bubble. Return the broth tube to the upright position, taking care to avoid reintroducing air into the Durham tube.

Using a sterile cotton swab, remove growth from the subculture and suspend it in sterile water to a density approximately equal to that of a McFarland no. 1 standard. Inoculate the medium with one drop of the standardized culture using a sterile 1 mL pipette.

Incubate the tubes at 25°C and examine at 5, 7, 10 and 14 days for growth and fermentation (gas production).

Expected Results
Growth is indicated by turbidity in the broth medium. Fermentation of the carbohydrate is indicated by accumulation of gas in the Durham tube and the change of the indicator to yellow.

References

1. Haley and Callaway. 1978. Laboratory methods in medical mycology, 4th ed. USDHEW Publication No. (CDC) 78-8361. CDC, Atlanta, Ga.
2. Warren and Shadomy. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Availability
BBL™ Yeast Fermentation Broth Base with Durham Tube
Cat. No. 297299 Prepared Tubes (K Tubes) – Pkg. of 10*

BBL™ Yeast Fermentation Broth with Carbohydrates and Durham Tube
Cat. No.
297302 Prepared Tubes with Dextrose (K Tubes) – Pkg. of 10*
297407 Prepared Tubes with Galactose (K Tubes) – Pkg. of 10*
297406 Prepared Tubes with Lactose (K Tubes) – Pkg. of 10*
297300 Prepared Tubes with Maltose (K Tubes) – Pkg. of 10*
297301 Prepared Tubes with Sucrose (K Tubes) – Pkg. of 10*
297337 Prepared Tubes with Trehalose (K Tubes) – Pkg. of 10*
297449 Prepared Tubes with Xylose (K Tubes) – Pkg. of 10*

**Store at 2-8°C.*

Yeast Media
Yeast Morphology Agar • Yeast Carbon Base • Yeast Nitrogen Base • Yeast Nitrogen Agar • Yeast Nitrogen Base w/o Amino Acids • Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Intended Use

Yeast Morphology Agar is used for classifying yeasts based on colonial characteristics and cell morphology.

Yeast Carbon Base is used for classifying yeasts based on nitrogen assimilation.

Yeast Nitrogen Base and Yeast Nitrogen Agar are used for classifying yeasts based on carbon assimilation.

Yeast Nitrogen Base without Amino Acids is used for classifying yeasts based on amino acid and carbohydrate requirements.

Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate is used for classifying yeasts based on carbon and nitrogen requirements.

Summary and Explanation

Yeasts are unicellular, eukaryotic, budding cells that are generally round-to-oval or elongate in shape.[1] They multiply principally by the production of blastoconidia (buds).[1] Yeast colonies are moist and creamy or glabrous to membranous in texture.[1] Yeasts are considered opportunistic pathogens.[1]

The yeast media cited are prepared according to the formulas of Wickerham.[2–6]

Yeast Carbon Base tests the ability of yeasts to assimilate nitrogen by the addition of various nitrogen sources. The inclusion of vitamins aids in the utilization of nitrogen-containing compounds by certain yeasts which cannot assimilate these compounds in the absence of vitamins.

Yeast Nitrogen Base is a suitable medium for studying strains of yeast that require certain vitamins.

Prepared plated Yeast Nitrogen Agar, which is Yeast Nitrogen Base plus 13.0 g/L of agar, is prepared according to Wickerham and Burton's formulation for use in an auxanographic technique for determining patterns of carbohydrate assimilation.[6] In the auxanographic technique originally devised by Beijerinck, small amounts of dry carbohydrates are placed on the surface of a heavily seeded synthetic agar medium.[7] Growth in the area surrounding a carbohydrate indicates that the yeast assimilated that sugar as a carbon source. The pattern of utilized carbohydrates is an auxanogram.

Alternate methods of applying the carbohydrates to the agar surface have been used. The dry carbohydrates used by Beijerinck may be replaced with filter-paper discs impregnated with carbohydrates (**Taxo**™ carbohydrate discs), by placing drops of carbohydrate solution onto the agar, or by placing the carbon sources in wells cut in the agar surface.[8]

Yeast Nitrogen Base without Amino Acids, which lacks the amino acids histidine, methionine and tryptophan, and Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate, which lacks amino acids and ammonium sulfate, are prepared according to Guenter's[9] modification of Wickerham's Yeast Nitrogen Base formulation.

These media are included in many applications for the study of yeasts in molecular genetics.[10,11]

Principles of the Procedure

Yeast Morphology Agar contains all essential nutrients and vitamins necessary for the cultivation of yeasts, including a source of carbohydrate.

Yeast Carbon Base contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of nitrogen.

Yeast Nitrogen Base contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of carbohydrate.

Prepared plated Yeast Nitrogen Agar is composed of a defined set of nutrients, including a nitrogen source, amino acids, minerals and vitamins required for the growth of yeasts, but without any energy source. This medium is used to determine the ability of a yeast species to utilize a carbohydrate that is added to the medium as the sole source of carbon.[8]

Yeast Nitrogen Base without Amino Acids contains all essential vitamins and inorganic salts necessary for the cultivation of yeasts except histidine, methionine, tryptophan and a source of carbohydrate.

Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate contains all essential nutrients and vitamins necessary for the cultivation of yeasts except amino acids and a source of nitrogen and carbohydrate.

User Quality Control

Identity Specifications

Difco™ Yeast Morphology Agar

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 3.5% solution, soluble in purified water upon boiling. Solution is very light amber, slightly opalescent.

Prepared Appearance: Very light amber, slightly opalescent without significant precipitate.

Reaction of 3.5% Solution at 25°C: pH 5.6 ± 0.2

Difco™ Yeast Carbon Base

Dehydrated Appearance: Off-white, free-flowing, homogeneous.

Solution: 1.17% (single-strength) and 11.7% (10×) solution, soluble in purified water with slight warming. Single-strength solution is colorless to very light amber, clear.

Prepared Appearance: Colorless to very light amber, clear.

Reaction of 1.17% Solution at 25°C: pH 5.5 ± 0.2

Difco™ Yeast Nitrogen Base

Dehydrated Appearance: Off-white, free-flowing, homogeneous.

Solution: 0.67% (single strength) and 6.7% (10×) solution, soluble in purified water with agitation. Single-strength solution is almost colorless and clear; 10× solution is yellow and clear.

Prepared Appearance: Colorless, clear.

Reaction of 0.67% Solution at 25°C: pH 5.4 ± 0.2

Difco™ Yeast Nitrogen Base without Amino Acids

Dehydrated Appearance: Off-white, free-flowing, homogeneous.

Solution: 0.67% (single strength) or 6.7% (10×) solution, soluble in purified water with agitation. Single-strength solution is colorless to very pale yellow and clear; 10× solution is yellow and clear.

Prepared Appearance: Colorless, clear.

Reaction of 0.67% Solution at 25°C: pH 5.4 ± 0.2

Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

Dehydrated Appearance: Light yellowish-beige, free-flowing, homogeneous.

Solution: 0.17% (single-strength) and 1.7% (10×) solution, soluble in purified water. Single-strength solution is colorless to very pale yellow and clear; 10× solution is yellow and clear.

Prepared Appearance: Colorless, clear.

Reaction of 0.17% Solution at 25°C: pH 4.5 ± 0.2

Cultural Response

Difco™ Yeast Morphology Agar

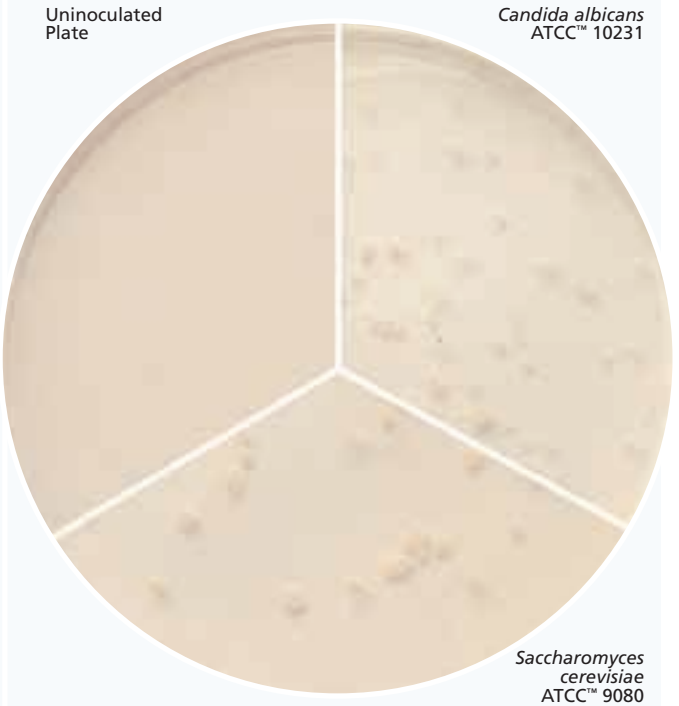
Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 25-30°C for 18-48 hours. Also, inoculate by the Dolman technique (streak and point) and add coverslips. Incubate at 25-30°C for 6-7 days and examine microscopically for hyphae.

ORGANISM	ATCC™	RECOVERY	DOLMAN PLATE TEST
Kloeckera apiculata	9774	Good	–
Saccharomyces cerevisiae	9080	Good	–
Candida albicans	10231	Good	Hyphae

Difco™ Yeast Carbon Base (with and without 5% ammonium sulfate) **Difco™ Yeast Nitrogen Base** (with and without 5% dextrose) **Difco™ Yeast Nitrogen Base without Amino Acids** (with and without 5% dextrose, 0.02% DL-methionine, 0.02% DL-tryptophan and 0.01% L-histidine) **Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate** (with and without 5% dextrose, 5% ammonium sulfate, 0.02% DL-methionine, 0.02% DL-tryptophan and 0.01% L-histidine)

Prepare the medium per label directions with and without the supplements indicated above. Add 1 mL of the filter-sterilized solution to 9 mL of sterile water, inoculate and incubate at 25-30°C for 2-5 days.

ORGANISM	ATCC™	GROWTH WITHOUT SUPPLEMENT(S)	GROWTH WITH SUPPLEMENT(S)
Kloeckera apiculata	9774	None to poor	Good
Saccharomyces cerevisiae	9080	None to poor	Good



Formulae

Difco™ Yeast Morphology Agar

Approximate Formula* Per Liter

Nitrogen Sources

Ammonium Sulfate	3.5	g
Asparagine	1.5	g

Carbon Source

Dextrose	10.0	g

Amino Acids

L-Histidine Monohydrochloride	10.0	mg
LD-Methionine	20.0	mg
LD-Tryptophan	20.0	mg

Vitamins

Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg

Compounds Supplying Trace Elements

Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg

Salts

Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g
Agar	18.0	g

Difco™ Yeast Carbon Base

Approximate Formula* Per Liter

Carbon Source

Dextrose	10.0	g

Amino Acids

L-Histidine Monohydrochloride	1.0	mg
LD-Methionine	2.0	mg
LD-Tryptophan	2.0	mg

Vitamins

Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg

Compounds Supplying Trace Elements

Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg

Salts

Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g

Difco™ Yeast Nitrogen Base

Approximate Formula* Per Liter

Nitrogen Source

Ammonium Sulfate	5.0	g

Amino Acids

L-Histidine Monohydrochloride	10.0	mg
LD-Methionine	20.0	mg
LD-Tryptophan	20.0	mg

Vitamins

Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg

Compounds Supplying Trace Elements

Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg

Salts

Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g

Difco™ Yeast Nitrogen Base without Amino Acids

Approximate Formula* Per Liter

Nitrogen Source

Ammonium Sulfate	5.0	g

Vitamins

Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg

Compounds Supplying Trace Elements

Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg

Salts

Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g

Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

Approximate Formula* Per Liter

Vitamins		
Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg
Compounds Supplying Trace Elements		
Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg
Salts		
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Yeast Morphology Agar

1. Suspend 35 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Yeast Carbon Base, Difco™ Yeast Nitrogen Base, Difco™ Yeast Nitrogen Base without Amino Acids or Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

1. To facilitate filtration, prepare a 10× solution as follows:
   Difco™ Yeast Carbon Base – Dissolve 11.7 g of base and a nitrogen source in 100 mL of purified water (with warming, if necessary). Mix well.
   Difco™ Yeast Nitrogen Base – Dissolve 6.7 g of base and 5 g of dextrose or equivalent amount of other carbohydrate in 100 mL of purified water (with warming, if necessary). Mix well.
   Difco™ Yeast Nitrogen Base without Amino Acids – Dissolve 6.7 g of base, 5 g of dextrose or equivalent amount of other carbohydrate and 5-10 mg% of the desired amino acid in 100 mL of purified water (with warming, if necessary). Mix well.
   Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate – Dissolve 1.7 g of base plus nitrogen and carbon sources as required in 100 mL of purifed water (with warming, if necessary). Mix well.
2. Filter sterilize.
3. Store at 2-8°C.
4. Prepare the final medium by aseptically pipetting 0.5 mL of the 10× solution into 4.5 mL of purified water. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Difco™ Yeast Morphology Agar

Inoculate plates using the Dolman technique. This is an excellent method for studying the hyphae of filamentous yeasts.

1. Near one side of the plate (from the relative positions of 10 o'clock to 2 o'clock), lightly inoculate a single streak taken from a slant culture.
2. In addition to the single streak, inoculate two points near the other side of the plate (at the 4 o'clock and 8 o'clock positions).
3. Cover a central section of the streak inoculation and one point inoculation with cover glasses, as follows:
   a. With forceps, remove a cover glass from absolute alcohol, drain momentarily, and burn off excess alcohol by passing over a low flame.
   b. When the cover glass has cooled, place one edge on the agar and allow it to fall across the central portion of the inoculated streak. Place a second cover glass over one point inoculation.
4. Incubate at 25-30°C for 6-7 days.
5. After incubation, observe with a high dry objective.

Difco™ Yeast Carbon Base, Yeast Nitrogen Base, Yeast Nitrogen Base without Amino Acids and Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

1. Inoculate the prepared tubed medium very lightly with the test organism.
2. Incubate at 25°C for 6-7 days.
3. After incubation (6-7 days and, if necessary, 20-24 days), shake the tubes to suspend growth.
4. Read for growth.

BBL™ Yeast Nitrogen Agar

1. Subculture the isolate to be identified onto a Sabouraud Dextrose Agar slant. Incubate at 30°C until good growth is observed (24-48 hours).
2. Using a sterile cotton swab, remove the growth from the subculture and suspend in 9 mL sterile water. Using a new sterile swab, uniformly inoculate the medium with the yeast suspension.
3. Following inoculation, place carbohydrate discs onto the surface of the medium. Press each disc with sterile forceps to make good contact with the agar surface.
4. Incubate the plates in an inverted position (agar side up) at 25°C for 48–72 hours.

Carbon Assimilation Test

Refer to the procedure described in the *Manual of Clinical Microbiology*.[8]

Nitrogen Assimilation Test

Refer to the procedure described in the *Manual of Clinical Microbiology*.[8]

Expected Results

Difco™ Yeast Morphology Agar

Using the high-dry objective, observe for hyphae of filamentous yeasts.

Difco™ Yeast Carbon Base, Yeast Nitrogen Base, Yeast Nitrogen Base without Amino Acids and Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

Measure growth turbidimetrically at 660 nm wavelength using a spectrophotometer. Turbidimetric readings on assay tubes should be comparable to the control.

BBL™ Yeast Nitrogen Agar

After sufficient incubation, a zone of growth should be visible in the area surrounding carbohydrates that have been assimilated. A yeast species may be presumptively identified based on a pattern of assimilation of carbohydrates. Consult appropriate texts for information on biochemical tests and other identification procedures to confirm findings.[8,12,13]

Limitation of the Procedure

Yeasts grown on a rich medium may carry a reserve of nitrogen in the form of protein. Possible errors due to this reserve are eliminated by making two serial transfers in the complete medium. When the first transfer is seven days old, the culture is shaken and one loopful is transferred to a second tube of the complete medium containing the same source of nitrogen. If a positive test is obtained when the second culture is seven days old, the organism being tested assimilates this particular nitrogen source.

References

1. Warren and Hazen. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. Wickerham. 1951. Taxonomy of yeasts. Technical bulletin No. 1029, U. S. Dept Agriculture, Washington, D.C.
3. Wickerham and Rettger. 1939. J. Tropical Med. Hyg. *42*:174.
4. Wickerham. 1946. J. Bacteriol. *52*:293.
5. Wickerham. 1943. J. Bacteriol. *46*:501.
6. Wickerham and Burton. 1948. J. Bacteriol. *56*:363.
7. Beijerinck. 1889. Arch. Neerl. Sci. Exactes Nat. *23*:367.
8. Warren and Shadomy. 1991. *In* Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.). Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
9. Guenter. Personal Communication.
10. Sherman, Fink and Hicks. 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
11. Brownstein, Silverman, Little, Burke, Korsmeyer, Schlessinger and Olson. 1989. Science. *244*:1348.
12. Haley, Trandel and Coyle. 1980. Cumitech 11, Practical method for culture and identification of fungi in the clinical mycology laboratory. Coord. ed., Sherris. American Society for Microbiology, Washington, D.C.
13. Larone. 1995. Medically important fungi: a guide to identification, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Yeast Morphology Agar

Cat. No. 239320 Dehydrated – 500 g

Difco™ Yeast Carbon Base

Cat. No. 239110 Dehydrated – 100 g

Difco™ Yeast Nitrogen Base

Cat. No. 239210 Dehydrated – 100 g

BBL™ Yeast Nitrogen Agar

Cat. No. 295977 Prepared Plates – Pkg. of 20*

Difco™ Yeast Nitrogen Base without Amino Acids

Cat. No. 291940 Dehydrated – 100 g
291920 Dehydrated – 2 kg
291930 Dehydrated – 10 kg

Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

Cat. No. 233520 Dehydrated – 100 g
233510 Dehydrated – 10 kg

**Store at 2-8°C.*

Yeast Mold (YM) Agar • Yeast Mold (YM) Broth

Intended Use

YM Agar and YM Broth are used for cultivating yeasts, molds and other aciduric microorganisms.

Summary and Explanation

YM Agar and YM Broth are prepared according to the formulae published by Wickerham.[1-3] Wickerham suggested that YM Broth acidified to pH 3.0-4.0 be used as an enrichment medium for yeasts from populations also containing bacteria and molds.

Media selectivity may be enhanced through acidification or through addition of selective agents. YM Broth may be acidified prior to sterilization. YM Agar should be sterilized without pH adjustment and sterile acid added to the sterile molten medium cooled to 45-50°C. Acidified YM Agar should not be heated. Antibiotics may be aseptically added to the sterile media. Other fungistatic materials, such as sodium propionate and diphenyl may be added to YM Agar to eliminate molds and permit the enumeration of yeasts in mixed populations.

Principles of the Procedure

Yeast extract is a source of trace elements, vitamins and amino acids. Malt extract is a source of carbon, protein and nutrients. Peptone is an additional source of carbon and provides nitrogen and amino acids. Dextrose provides carbon. Agar is the solidifying agent.

Formulae

Difco™ YM Agar

Approximate Formula* Per Liter

Yeast Extract	3.0	g
Malt Extract	3.0	g
Peptone	5.0	g
Dextrose	10.0	g
Agar	20.0	g

Difco™ YM Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

Identity Specifications

Difco™ YM Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 6.2 ± 0.2

Difco™ YM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent. At pH adjusted to 3.0-4.0, medium becomes slightly opalescent.
Reaction of 2.1% Solution at 25°C:	pH 6.2 ± 0.2

Cultural Response

Difco™ YM Agar or YM Broth

Prepare two sets of agar plates or broth tubes (one set pH 6.2, one set adjusted to pH 3.0-4.0) per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY pH 3.0-4.0	RECOVERY pH 6.2
Aspergillus brasiliensis (niger)	16404	10^2-10^3	Good	Good
Candida albicans	10231	10^2-10^3	Good	Good
Escherichia coli	25922	10^2-3×10^2	Marked to complete inhibition	Good
Lactobacillus rhamnosus	7469	10^2-3×10^2	Poor to fair	Good
Saccharomyces cerevisiae	9763	10^2-10^3	Good	Good

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ YM Agar – 41 g;
   Difco™ YM Broth – 21 g.
   Mix thoroughly.
2. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave the agar and broth media at 121°C for 15 minutes.
4. To increase selectivity, acidify the medium to pH 3.0 to 4.0 (by adding sterile 10% HCl, tartaric acid or 10% citric acid) or add antibiotics (penicillin 20 units per mL final concentration or streptomycin 40 µg per mL final concentration) using aseptic technique. Acidified agar medium should not be reheated.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate YM Agar plates or YM Broth tubes with sample to evaluate for the presence of yeasts, molds, or aciduric microorganisms. Incubate at 30 ± 2°C for 18-72 hours.

To favor isolation of fermentative species, add a layer of sterile paraffin oil 1 cm deep on the surface of the inoculated broth. Incubate the culture until growth appears and then streak onto YM Agar to obtain isolated yeast colonies. To isolate fermentative and oxidative strains, place acidified inoculated YM Broth on a rotary shaker for 1 or 2 days. This favors yeast recovery while preventing the sporulation of molds.

Expected Results

Examine the plates or tubes for growth. Record YM Agar results as colony-forming units (CFU) per volume of sample. Record YM Broth results as growth or no growth.

References

1. Wickerham. 1939. J. Tropical Med. Hyg. *42*:176.
2. U. S. Department of Agriculture. 1951. Tech. Bull. No. 1029.
3. Jong and Edwards. 1991. American Type Culture Collection catalog of filamentous fungi, 18th ed. American Type Culture Collection, Rockville, Md.

Availability

Difco™ YM Agar

AOAC COMPF

Cat. No. 271210 Dehydrated – 500 g

Difco™ YM Broth

Cat. No. 271120 Dehydrated – 500 g

Yersinia Selective Agar Base (CIN Agar Base) Yersinia Antimicrobic Supplement CN

(See CIN Agar Base)

Section IV

Reference Guide

Culture Media for Specific Groups of Microorganisms

ORGANISMS	ISOLATION	CULTIVATION	IDENTIFICATION	MAINTENANCE
Actinomycetes (aerobic) *Nocardia* *Streptomyces* *Rhodococcus*	Actinomycete Isolation Agar Brain Heart CC Agar Columbia CNA Agar Lowenstein-Jensen Media Middlebrook 7H10 Agar Blood Agar Chocolate Agar Sabouraud Dextrose Agar with and without CC Tryptic/**Trypticase**™ Soy Media	Bushnell-Haas Medium Czapek Solution Agar Eugon Agar ISP Medium 1,2, and 4 Sabouraud Dextrose Agar Tryptic/**Trypticase** Soy Agar YM Agar/YM Broth	Casein Agar CTA Media Indole Nitrite Medium ISP Medium 4 Nocardia QUAD ID Nutrient Gelatin Tyrosine Agar Xanthine Agar	Brain Heart Infusion Agar Chocolate Agar **CTA Medium**™ ISP Medium 4 Tryptic/**Trypticase** Soy Agar YM Agar/YM Broth
Anaerobes (exclusive of clostridia): *Actinomyces* *Bacteroides* *Bifidobacterium* *Eubacterium* *Fusobacterium* *Lactobacillus* *Peptostreptococcus* *Porphyromonas* *Prevotella* *Proprionibacterium* *Veillonella*	Anaerobic Agar Bacteroides Bile Esculin Agar Brewer Anaerobic Agar Brucella Laked Blood Agar with KV CDC Anaerobe Blood Agar CDC Anaerobe Blood Agar with KV CDC Anaerobe Blood Agar with PEA CDC Anaerobe Laked Blood Agar with KV Columbia Anaerobic Sheep Blood Agar Cooked Meat Medium Liver Infusion Agar/Broth Liver Veal Agar Schaedler Agar/Broth Sulfite Agar Thioglycollate Media Tryptic/**Trypticase** Soy Media	Actinomyces Broth Anaerobic Agar Brewer Anaerobic Agar CDC Anaerobe Blood Agar Columbia Anaerobic Sheep Blood Agar Cooked Meat Medium Liver Veal Agar Schaedler Agar/Broth Sulfite Agar Thioglycollate Media Tryptic/**Trypticase** Soy Media	Bacteroides Bile Esculin Agar Cooked Meat Medium **CTA Medium** with Carbohydrates Indole Nitrite Medium Sulfite Agar Thioglycollate Media	Cooked Meat Medium **CTA Medium** Thioglycollate Medium without Indicator-135C
Bordetella	Bordet Gengou Agar Base Charcoal Agar Chocolate Agar Regan-Lowe Charcoal Agar Base	Bordet Gengou Agar Base Charcoal Agar Chocolate Agar	Blood Agar Heart Infusion Agar MacConkey Agar Litmus Milk Motility Test Medium Nitrate Broth Urea Agar Base Urease Test Broth	Bordet Gengou Agar Base
Brucella	Brucella Agar/Broth Eugon Agar Liver Infusion Agar/Broth Tryptic/**Trypticase** Soy Media Tryptose Broth	Brucella Agar/Broth Eugon Agar Eugon Broth/**Eugonbroth**™ Liver Infusion Agar/Broth Potato Infusion Agar Tryptic/**Trypticase** Soy Media	**CTA Medium** Urea Agar Base Urease Test Broth	**CTA Medium** Nutrient Agar
Campylobacter	Brucella Agar/Broth Campy CSM Agar Campy CVA Agar Campy-Cefex Agar Campylobacter Agar Base Campylobacter Agar with 5 Antimicrobics and 10% SB Campylobacter Thioglycollate with 5 Antimicrobics	Campy CSM Agar Campy CVA Agar Campy-Cefex Agar Campylobacter Agar Base Campylobacter Agar with 5 Antimicrobics and 10% SB	Campy CSM Agar Campy CVA Agar Campylobacter Agar Base Campylobacter Agar with 5 Antimicrobics and 10% SB Nutrient Broth for Motility Triple Sugar Iron (TSI) Agar for H_2S	
Clostridium	Anaerobic Agar CDC Anaerobe Blood Agar CDC Anaerobe Blood Agar with PEA Clostridium Difficile Selective Agar Cooked Meat Medium Lecithin Lactose Agar Liver Infusion Agar/Broth McClung Toabe Agar Reinforced Clostridial Medium SFP Agar SPS Agar Schaedler Agar/Broth TSN Agar Thioglycollate Media	Anaerobic Agar CDC Anaerobe Blood Agar Columbia Anaerobic Blood Agar Cooked Meat Medium Differential Reinforced Clostridial Agar Egg Yolk Agar, Modified McClung Toabe Agar Reinforced Clostridial Medium SFP Agar SPS Agar Schaedler Agar/Broth Thioglycollate Media **Trypticase** Soy Broth with 0.1% Agar	Clostridium Difficile Selective Agar Cooked Meat Medium Differential Reinforced Clostridial Agar Egg Yolk Agar, Modified Indole Nitrite Medium Lecithin Lactose Agar Litmus Milk McClung Toabe Agar SFP Agar SPS Agar Sulfite Agar Thioglycollate Media Tryptic/**Trypticase** Agar Base	Cooked Meat Medium Thioglycollate Medium without Indicator-135C Tryptic/**Trypticase** Agar Base

Culture Media for Specific Groups of Microorganisms

ORGANISMS	ISOLATION	CULTIVATION	IDENTIFICATION	MAINTENANCE
Coliforms and *Proteus*	A-1 Medium Brilliant Green Bile Agar Brilliant Green Bile Broth, 2% Brilliant Green Bile Broth, 2% with MUG **BBL**™ **CHROMagar**™ O157 **BBL CHROMagar** Orientation CLED Agar EC Medium/Broth EC Medium/Broth with MUG EC Medium, Modified Endo Agar m Endo Agar LES m Endo Broth MF™ EMB, Levine EMB, Modified m FC Broth Hektoen Enteric Agar Lactose Broth Lauryl Sulfate/Tryptose Broth Lauryl Sulfate/Tryptose Broth with MUG MI Agar MacConkey Media MacConkey Sorbitol Media Nutrient Agar with MUG Presence-Absence Broth R2A Agar Salmonella Shigella Agar Sorbitol MacConkey with CT TAT Broth Base m TEC Agar Modified m TEC Agar Tryptone Water Violet Red Bile Agar with and without MUG XL Agar Base/XLD Agar	Brain Heart Infusion Brain Heart Infusion Agar MacConkey Agar Tryptic/**Trypticase** Soy Media	A-1 Broth Acetate Differential Agar Brilliant Green Bile Agar Brilliant Green Bile Broth, 2% **BBL CHROMagar** O157 **BBL CHROMagar** Orientation Decarboxylase Media EC Medium/Broth EC Medium/Broth with MUG EC Medium, Modified Endo Agar m Endo Agar LES m Endo Broth MF™ EMB, Levine EMB, Modified Enteric Fermentation Base Hektoen Enteric Agar Indole Nitrite Medium Kligler Iron Agar Koser Citrate Lauryl Sulfate Broth/Lauryl Tryptose Broth with MUG MI Agar MacConkey Media MacConkey Sorbitol Media Malonate Broth, Ewing Modified MR-VP Broth Minerals Modified Glutamate Broth MIO Medium Motility Test Medium Nitrate Broth Nutrient Agar with MUG Nutrient Gelatin OF Basal Medium Phenol Red Broth with Carbohydrates Phenylalanine Agar Purple Broth Base Salmonella Shigella Agar SIM Medium Simmons Citrate Agar m TEC Agar Modified m TEC Agar Triple Sugar Iron (TSI) Agar Tryptic/**Trypticase** Agar Base with **Taxo**™ Discs Tryptophan 1% Solution Urea Agar Base Urease Test Broth Violet Red Bile Agar Violet Red Bile Agar with MUG XL Agar Base/XLD Agar	Nutrient Agar Tryptic/**Trypticase** Soy Media
Corynebacterium	Phenylethyl Alcohol Agar Serum Tellurite Agar Tinsdale Base Tinsdale Enrichment, Desiccated	Brain Heart Infusion Agar Tryptic/**Trypticase** Soy Agar	CTA Agar Indole Nitrite Medium Nitrate Broth Serum Tellurite Agar Starch Agar Urea Agar	Blood Agar Base with 5% Rabbit Blood **CTA Medium** Nutrient Agar Tryptic/**Trypticase** Soy Agar

Culture Media for Specific Groups of Microorganisms

ORGANISMS	ISOLATION	CULTIVATION	IDENTIFICATION	MAINTENANCE
Enteric Bacilli	Bismuth Sulfite Agar DCLS Agar Desoxycholate Agar Desoxycholate-Citrate Agar Desoxycholate Lactose Agar Endo Agar EMB, Levine EMB, Modified GN Broth Hektoen Enteric Agar MacConkey Media MacConkey Sorbitol Media Salmonella Shigella Agar TAT Broth Base Tryptic/**Trypticase** Soy Agar Violet Red Bile Agar XL Agar Base/XLD Agar	Brain Heart Infusion Brain Heart Infusion Agar Tryptic/**Trypticase** Soy Media	Bismuth Sulfite Agar **BBL CHROMagar** Orientation Desoxycholate Agar Desoxycholate-Citrate Agar Desoxycholate Lactose Agar DNase Test Agar with Toluidine Blue Decarboxylase Media Endo Agar EMB, Levine EMB, Modified Hektoen Enteric Agar Indole Nitrite Medium Kligler Iron Agar Lysine Iron Agar MacConkey Media MacConkey Sorbitol Media Malonate Broth, Ewing Modified Moeller KCN Broth Base MIO Medium Motility Test Medium Nitrate Broth Nutrient Gelatin OF Basal Medium Phenol Red Media Phenylalanine Agar Purple Broth Base Salmonella Shigella Agar SIM Medium Simmons Citrate Agar Triple Sugar Iron (TSI) Agar Tryptophan 1% Solution Urea Agar Base Urease Test Broth Violet Red Bile Agar XL Agar Base/XLD Agar	Nutrient Agar Tryptic/**Trypticase** Soy Media
Enterococci Group D Streptococci	Azide Dextrose Broth Bile Esculin Agar Columbia CNA Agar *M*-E Agar Base mEI Agar **Enterococcosel**™ Agar Esculin Iron Agar Ethyl Violet Azide (EVA) Broth KF Streptococcal Agar/Broth m-Enterococcus Agar MacConkey Agar without CV Phenylethyl Alcohol Agar SF Broth/Medium	Brain Heart Infusion Brain Heart Infusion Agar SF Broth/Medium Tryptic/**Trypticase** Soy Media	Bile Esculin Agar **CTA Medium** w/Carbohydrates mEI Agar **Enterococcosel** Agar/Broth KF Streptococcal Agar /Broth MacConkey Agar without CV Salt Broth, Modified SF Broth/Medium **Trypticase** Soy Broth with 6.5% NaCl	Brain Heart Infusion **CTA Medium** Stock Culture Agar **Trypticase** Soy Agar with 5% Sheep Blood

Culture Media for Specific Groups of Microorganisms

ORGANISMS	ISOLATION	CULTIVATION	IDENTIFICATION	MAINTENANCE
Fungi (Yeasts and Molds)	BiGGY Agar Brain Heart CC Agar Brain Heart Infusion Agar Candida BCG Agar **BBL CHROMagar** Candida Cooke Rose Bengal Agar Corn Meal Agar DRBC Agar Dermatophyte Test Medium Dextrose Agar Fluid Sabouraud Medium HC Agar Base Inhibitory Mold Agar M-Green Yeast and Mold Broth Malt Agar Malt Extract Agar/Broth Mycological Agar **Mycophil**™ Agar/Broth **Mycophil** Agar with Low pH **Mycosel**™ Agar Oatmeal Agar OGYE Agar Base Orange Serum Agar Petragnani Medium **Phytone**™ Yeast Extract Agar Potato Dextrose Agar Rose Bengal Agar Sabouraud Brain Heart Infusion Agar Sabouraud Media Special Yeast and Mold Medium YM Agar/Broth Yeast Extract Glucose Chloramphenicol Agar	Brain Heart Infusion Agar/Broth Corn Meal Agar Czapek-Dox Broth Czapek Solution Agar Malt Agar Malt Extract Agar/Broth **Mycophil**™ Agar/Broth Oatmeal Agar OGYE Agar Base Potato Dextrose Agar/Broth Sabouraud Media Special Yeast and Mold Medium Tryptic/**Trypticase** Soy Media YM Agar/Broth Yeast Extract Glucose Chloramphenicol Agar Yeast Morphology Agar	BiGGY Agar Candida BCG Agar **BBL CHROMagar** Candida Corn Meal Agar DRBC Agar Rice Extract Agar Rose Bengal Agar Trichophyton Agars 1-7 Yeast Extract Glucose Chloramphenicol Agar Yeast Nitrogen Base without Amino Acids Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate Yeast Morphology Agar Yeast Nitrogen Base	**CTA Medium** **Mycophil** Agar Potato Dextrose Agar Sabouraud Dextrose Agar Tryptic/**Trypticase** Agar Base YPD Agar/Broth
Gardnerella vaginalis	HBT Bilayer Medium V Agar	Chocolate Agar Columbia CNA Agar Casman Agar Base with 5% Rabbit Blood	HBT Bilayer Medium Sodium Hippurate Broth Starch Agar with Bromcresol Purple V Agar	Casman Agar Base with 5% Rabbit Blood
Haemophilus	Casman Agar Base Chocolate II Agar GC Medium Base	Casman Agar Base Chocolate II Agar GC Medium Base	Hemo QUAD ID Tryptic/**Trypticase** Soy Agar with **Taxo** X, V and XV Factor Strips	Chocolate II Agar **CTA Medium**
Lactobacillus *Leuconostoc*	APT Agar/Broth Eugon Agar Lactobacillus MRS Agar/Broth LBS Agar/Broth MRS Agar/Broth Orange Serum Agar Raka Ray No. 3 Broth/Medium Reinforced Clostridial Medium Snyder Test Agar Thayer Martin Agar	APT Agar/Broth Chocolate II Agar Elliker Broth Eugon Agar LBS Agar/Broth MRS Agar/Broth Rogosa SL Agar/Broth Tomato Juice Agar Tomato Juice Agar, Special **Trypticase** Soy Agar with 5% Sheep Blood Universal Beer Agar (UBA)	Esculin Agar Slants Indole Nitrite Medium Litmus Milk Moeller Decarboxylase Broth with Arginine MRS Broth **Trypticase** Soy Broth with 6.5% NaCl	**CTA Medium** Eugon Agar Lactobacilli MRS Broth Tomato Juice Agar
Legionella	BCYE Agar Base BCYE Agar BCYE Differential Agar BCYE Selective Agar with PAC BCYE Selective Agar with PAV BCYE Selective Agar with CCVC Legionella Agar Base Legionella Agar Enrichment	BCYE Agar	BCYE Agar BCYE Differential Agar Sodium Hippurate Broth	BCYE Agar

Culture Media for Specific Groups of Microorganisms

ORGANISMS	ISOLATION	CULTIVATION	IDENTIFICATION	MAINTENANCE
Listeria	Buffered Listeria Enrichment Broth **BBL CHROMagar** Listeria Demi-Fraser Broth Fraser Broth GBNA Medium Listeria Enrichment Broth Listeria Enrichment Broth, Modified LPM Agar Base Oxford Medium Base PALCAM Medium Base Phenylethyl Alcohol Agar Tryptic/**Trypticase** Soy Agar UVM Modified Listeria Enrichment Broth Universal Preenrichment Broth	Eugon Agar Listeria Enrichment Broth LPM Agar Base PALCAM Medium Base Thioglycollate Medium without Indicator-135C **Trypticase** Soy Agar with 5% Sheep Blood	**BBL CHROMagar** Listeria **CTA Medium** Esculin Agar Slant Indole Nitrite Medium Litmus Milk MR-VP Broth Motility Test Medium Oxford Medium Base Purple Broth Base Triple Sugar Iron (TSI) Agar	Brain Heart Infusion Agar **CTA Medium** Tryptic/**Trypticase** Soy Agar
Mycobacterium	Dubos Oleic Agar Base Lowenstein-Jensen Media Middlebrook and Cohn 7H10 Agar Base Mycobacteria 7H11 Agar **Mycobactosel** L-J Medium Petragnani Medium Selective 7H11 Agar Seven H11 Agar Base Herrold's Egg Yolk Agar with ANV and Mycobactin J Herrold's Egg Yolk Agar with ANV, without Mycobactin J Wallenstein Medium	Dubos Broth Base Dubos Broth, Enriched Lowenstein-Jensen Media Middlebrook 7H9 Broth Base Middlebrook and Cohn 7H10 Agar Base Mycobacteria 7H11 Agar Petragnani Medium Seven H11 Agar Base Wallenstein Medium	Arylsulfatase Broth Lowenstein Jensen Medium Lowenstein Jensen with 5% NaCl MacConkey Agar without CV Middlebrook 7H9 Broth Urea Agar Base	Lowenstein-Jensen Media Middlebrook and Cohn 7H10 Agar Base Petragnani Medium
Mycoplasma *Ureaplasma*	Mycoplasma Broth Base (Frey) PPLO Agar PPLO Broth without CV	A7 Agar, Modified Mycoplasma Broth Base (Frey) PPLO Agar PPLO Broth without CV	A7 Agar, Modified	Mycoplasma Agar Base
Neisseria	Casman Agar Base Chocolate II Agar GC Medium Base **GC-Lect**™ Agar Eugon Agar Martin-Lewis Agar Modified Thayer-Martin (MTM II) Agar Thayer-Martin Selective Agar Tryptose Phosphate Broth	Brain Heart Infusion Agar Casman Agar Base Chocolate II Agar Eugon Agar Eugon Broth/**Eugonbroth** GC Medium Base Tryptose Phosphate Broth	**CTA Medium** with Carbohydrates Rapid Fermentation Medium	Chocolate II Agar **CTA Medium**
Pseudomonas *Acinetobacter* *Alcaligenes* *Burkholderia* and other Nonfermenters	*M*-PA-C Agar **Pseudosel**™ (Cetrimide) Agar PC (*Burkholderia cepacia*) Agar Pseudomonas Isolation Agar	Eugon Agar Tryptic/**Trypticase** Soy Agar	Acetamide Agar Gelatin MacConkey Agar Moeller Decarboxylase Broths Nitrate Broth OF Basal Medium Phenol Red Media Pseudomonas F and P Agars Triple Sugar Iron (TSI) Agar	Nutrient Agar Tryptic/**Trypticase** Agar Base

Culture Media for Specific Groups of Microorganisms

ORGANISMS	ISOLATION	CULTIVATION	IDENTIFICATION	MAINTENANCE
Salmonella *Shigella*	BG Sulfa Agar Bismuth Sulfite Agar Brilliant Green Agar Brilliant Green Agar Modified m Brilliant Green Broth Buffered Peptone Water Buffered Peptone Casein Water **BBL CHROMagar** Salmonella DCLS Agar Desoxycholate Agar Desoxycholate-Citrate Agar Desoxycholate Lactose Agar Endo Agar EMB, Levine EMB, Modified GN Broth/GN Broth Hajna Hektoen Enteric Agar Lactose Broth M Broth MacConkey Media Muller Kauffmann Tetrathionate Broth Base Rappaport-Vassiliadis Medium Rappaport-Vassiliadis R10 Broth RVS Soy Broth SBG Sulfa Enrichment Salmonella Shigella Agar Selenite Cystine Broth Selenite Broth/Selenite-F Broth Shigella Broth Tetrathionate Broth Base Universal Preenrichment Broth VJ (Vogel & Johnson) Agar XL Agar Base/XLD Agar XLT4 Agar Base w/Supplement	Brain Heart Infusion Brain Heart Infusion Agar Tryptic/**Trypticase** Soy Media	Acetate Differential Agar BG Sulfa Agar Bismuth Sulfite Agar Brilliant Green Agar Brilliant Green Agar Modified **BBL CHROMagar** Salmonella DCLS Agar Decarboxylase Media Desoxycholate Agar Desoxycholate-Citrate Agar Desoxycholate Lactose Agar Endo Agar EMB, Levine EMB, Modified Hektoen Enteric Agar Indole Nitrite Medium Kligler Iron Agar Lysine Iron Agar MacConkey Media Malonate Broth, Ewing Modified Motility Test Medium Nitrate Broth Nutrient Gelatin OF Basal Medium Phenol Red Media Purple Broth Base Rappaport-Vassiliadis Medium Salmonella Shigella Agar SIM Medium Simmons Citrate Agar Triple Sugar Iron (TSI) Agar Tryptophan 1% Solution Urea Agar Base Urease Test Broth XL Agar Base/XLD Agar XLT4 Agar Base with Supplement	Nutrient Agar Tryptic/**Trypticase** Soy Media
Staphylococci	Baird-Parker Agar Base Chapman Stone Agar **BBL CHROMagar** MRSA **BBL CHROMagar** Staph aureus Coagulase Mannitol Agar Columbia CNA Agar Giolitti-Cantoni Broth Base Mannitol Salt Agar Phenylethyl Alcohol Agar Staphylococcus Medium 110 Tellurite Glycine Agar VJ (Vogel & Johnson) Agar	Tryptic/**Trypticase** Soy Media	Baird-Parker Agar Base Chapman Stone Agar **BBL CHROMagar** MRSA **BBL CHROMagar** Staph aureus Coagulase Mannitol Agar Columbia CNA Agar **CTA Medium** with Mannitol DNase Test Agar DNase Test Agar with Methyl Green DNase Test Agar with Toluidine Blue Mannitol Salt Agar Phenol Red Mannitol Broth Spirit Blue Agar Staphylococcus Medium 110 Tellurite Glycine Agar	**CTA Medium** Nutrient Agar **Trypticase** Soy Agar

Culture Media for Specific Groups of Microorganisms

ORGANISMS	ISOLATION	CULTIVATION	IDENTIFICATION	MAINTENANCE
Streptococcus (Non-Group D)	Azide Blood Agar Base Bile Esculin Agar Columbia CNA Agar **ssA**™ Agar (Group A Selective Strep Agar with 5% SB) M17 Broth Mitis Salivarius Agar Neomycin Blood Agar Phenylethyl Alcohol Agar SXT Blood Agar Todd Hewitt Broth Tryptose Phosphate Broth	Brain Heart Infusion Brain Heart Infusion Agar Todd Hewitt Broth Tryptic/**Trypticase** Soy Media Tryptose Phosphate Broth	Azide Blood Agar Base Bile Esculin Agar Columbia CNA Agar Moeller Decarboxylase Broth with Arginine MR-VP Broth Sodium Hippurate Broth Stock Culture Agar **Trypticase** Soy Agar with 5% Sheep Blood **Trypticase** Soy Broth with 6.5% NaCl	Brain Heart Infusion Broth **CTA Medium** **Trypticase** Soy Agar with 5% Sheep Blood
Trichomonas	**Trichosel**™ Broth, Modified			
Vibrio	Alkaline Peptone Water DCLS Agar TCBS Agar	**Trypticase** Soy Agar with 5% Sheep Blood	DCLS Agar Kligler Iron Agar Moeller Decarboxylase Broths MR-VP Broth Purple Agar/Broth Base Simmons Citrate	Brain Heart Infusion Agar Marine Agar 2216 Nutrient Agar
Yersinia enterocolitica	CIN Agar Yersinia Selective Agar Base MacConkey Agar	**Trypticase** Soy Agar with 5% Sheep Blood MacConkey Agar	CIN Agar Yersinia Selective Agar Base Decarboxylase Broths Kligler Iron Agar Motility Test Medium MR-VP Broth Simmons Citrate Triple Sugar Iron (TSI) Agar	Brain Heart Infusion Agar Nutrient Agar **Trypticase** Soy Agar with 5% Sheep Blood Tryptose Agar

Agar Selection Guide

	PRODUCTS						
	BACTO™ AGAR	AGAR, GRADE A	AGAR, GRANULATED	AGAR, TECHNICAL	AGAR, NOBLE	AGAROSE	AGAR, SELECT
BACTERIOLOGICAL APPLICATIONS							
Auxotrophic studies	++	++	+				
Bacteriology, research	++	++	+				
Bacteriology, general purpose	++	++	+	+			
Bacteriophage studies	++	+					
Biotechnology production	+	+	++	+/-			
General microbial production	+	+	++	+/-			
Growth of fastidious organisms	++	++					
Identification of pathogenic organisms	++	++	+	+/-			
Microaerophilic studies	++	+					
Molecular genetics	++	++	+				++
Prepared plate manufacture	+	+	++				
Quality control, production	++	+	+	+/-			
Quality control, environmental	+	+	++	+/-			
Transformation of bacteria	++	+					
Transformation of yeast	++	+					
NON-BACTERIOLOGICAL APPLICATIONS							
Immunodiffusion					++	++	
Electrophoresis					++	++	
Tissue Culture, mammalian					++		
Tissue Culture, plant	++	++			+		
Histology, tissue embedding	+	+			++		
Histology, bone marrow embedding	++	+			+		
Insect growth substrate				++			

Key
++ Recommended
+ Suitable
+/– Marginal

Antimicrobial Effectiveness Testing[1]

	BIOCIDE CLASS						
	MERCURIALS	ALDEHYDES, HALOGENS, MERCURIALS	GLUTERALDEHYDES, MERCURIALS	QUARTERNARY AMMONIUM COMPOUNDS (QACS), PARAHYDROXYBENZOATES (PARABENS), BIS-BIGUANIDES	QACS, IODINE, PARABENS	PHENOLICS, ALCOHOL, ALDEHYDES, SORBATE
POTENTIAL NEUTRALIZING AGENTS/METHOD	**SODIUM THIOGLYCOLLATE**	**SODIUM THIOSULFATE**	**SODIUM BISULFITE**	**LECITHIN**	**POLYSORBATE 80**	**DILUTION**
PRODUCTS						
D/E Neutralizing Agar	•	•	•	•	•	•
D/E Neutralizing Broth	•	•	•	•	•	•
Letheen Agar				•	•	•
Letheen Agar, Modified			•	•	•	•
Letheen Broth				•	•	•
Letheen Broth, Modified			•	•	•	•
Microbial Content Test Agar (TSA with Lecithin & Polysorbate 80)				•	•	•
Neutralizing Buffer		•		• (aryl sulfonate)		•
Sabouraud Dextrose Agar with Lecithin & Polysorbate 80				•	•	•
Standard Methods Agar with Lecithin & Polysorbate 80				•	•	•
TAT Broth Base				•	•	•
Thioglycollate Media	•					•
Tryptic/**Trypticase**™ Soy Agar with Lecithin & Polysorbate 80				•	•	•

Reference
1. United States Pharmacopeial Convention, Inc. 2008. The United States Pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Antimicrobial Residue Testing

	TEST METHODS							
	SWAB TEST ON PREMISES (STOP)[1]	CALF ANTIBIOTIC AND SULFONAMIDE TEST (CAST)[1]	SULFONAMIDE RESIDUES TEST[1]	FAST ANTIMICROBIAL SCREEN TEST (FAST)[1]	MODIFIED FAST ANTIMICROBIAL SCREEN TEST (M-FAST)[1]	BIOASSAY FOR ANTIMICROBIAL RESIDUES[1]	QUANTITATIVE DETECTION OF PENICILLIN[2,3]	QUANTITATIVE DETECTION OF ANTIBIOTICS[3]
AGRICULTURAL SOURCES	**ANIMAL CARCASSES**	**CALVES**	**MEAT/ POULTRY**	**BOVINE CARCASSES**	**BOVINE CARCASSES**	**MEAT/ POULTRY**	**MILK**	**ANIMAL FEED**
PRODUCTS								
AK Agar #2 (Sporulating Agar)	•	•	•	•	•	•		
Antibiotic Medium 1						•	•	•
Antibiotic Medium 2						•		•
Antibiotic Medium 3								•
Antibiotic Medium 4							•	•
Antibiotic Medium 5	•					•		•
Antibiotic Medium 8						•		•
Antibiotic Medium 11						•		•
Antibiotic Medium 19								•
Brain Heart Infusion Broth	•	•	•	•	•	•		
Columbia Blood Agar Base	•	•	•	•	•			
Mannitol Egg Yolk Polymyxin (MYP) Agar						•		
Mueller Hinton Agar	•	•	•	•	•			
Noble Agar		•						
Penicillinase							•	
Plate Count Agar	•	•	•	•	•	•		
Trypticase™/Tryptic Soy Agar						•	•[2]	
Trypticase™ Soy Agar with 5% Sheep Blood						•		
Trypticase™/Tryptic Soy Broth							•[3]	
Tryptic Soy Broth without Dextrose							•	

References
1. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Bionutrient Selection Guide

PRODUCTS	BIONUTRIENT MEDIA PRODUCTS	PEPTONES FOR CELL CULTURE	PEPTONES FOR FERMENTATION
	APPLICATIONS		
Acidicase™ Peptone, **BBL**™			•
BD Cell™ MAb Medium, Animal Component Free	•		
BD Cell™ MAb Medium, Quantum Yield	•		
BD Cell™ MAb Medium, Serum Free	•		
Beef Extract, Desiccated, **Bacto**™			•
Beef Extract, Powder, **BBL**™			•
Biosate™ Peptone, **BBL**™			•
Casamino Acids, **Bacto**™			•
Casamino Acids, Technical, **Bacto**™			•
Casein Digest, **Difco**™			•
Casitone, **Bacto**™			•
Gelysate™ Peptone, **BBL**™			•
M9 Minimal Salts 5x, **Difco**™	•		
Malt Extract, **Bacto**™			•
Neopeptone, **Bacto**™			•
Peptone, **Bacto**™			•
Phytone™ Peptone, **BBL**™		•	•
Polypeptone™ Peptone, **BBL**™			•
Proteose Peptone, **Bacto**™			•
Proteose Peptone, **BiTek**™			•
Proteose Peptone No. 2, **Bacto**™			•
Proteose Peptone No. 3, **Bacto**™			•
Proteose Peptone No. 3, **BiTek**™			•

PRODUCTS	BIONUTRIENT MEDIA PRODUCTS	PEPTONES FOR CELL CULTURE	PEPTONES FOR FERMENTATION
	APPLICATIONS		
Proteose Peptone No. 4, **Bacto**™			•
Select APS™ LB Broth Base, **BBL**™	•		
Select APS™ Super Broth, **Difco**™	•		
Select Phytone™ UF, **Difco**™		•	•
Select Soytone, **Difco**™		•	•
Soytone, **Bacto**™			•
TC Lactalbumin Hydrolysate, **Bacto**™			•
TC Yeastolate, **Bacto**™		•	•
TC Yeastolate, UF, **Difco**™		•	•
Trypticase™ Peptone, **BBL**™			•
Tryptone, **Bacto**™			•
Tryptone, **BiTek**™			•
Tryptose, **Bacto**™			•
Yeast Extract, **Bacto**™		•	•
Yeast Extract, **BBL**™			•
Yeast Extract, LD, **Difco**™			•
Yeast Extract, Technical, **Bacto**™			•
Yeast Extract, UF, **Difco**™		•	•
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate, **Difco**™	•		
Yeast Nitrogen Base w/o Amino Acids, **Difco**™	•		
Yeast Nitrogen Base, **Difco**™	•		

Cosmetic Testing

PRODUCTS	REFERENCE METHODS	APPLICATIONS: ANAEROBES	CANDIDA ALBICANS	CLOSTRIDIA	DILUENTS	ENTEROBACTERIACEAE	ESCHERICHIA COLI	GRAM-NEGATIVE SCREENING	GRAM-NEGATIVE NONFERMENTATIVE BACILLI	GRAM-POSITIVE BACTERIA	NEUTRALIZATION OF ANTIMICROBICS	PSEUDOMONAS AERUGINOSA	SALMONELLA	STANDARD PLATE COUNT	STAPHYLOCOCCUS AUREUS	YEAST & MOLD
Acetimide Agar	BAM								•							
Blood Agar Base	BAM	•								•						•
Brain Heart Infusion	BAM									•						
Brain Heart Infusion Agar	BAM									•					•	
Buffered Sodium Chloride-Peptone Solution pH 7.0	ISO, USP				•											
CDC Anaerobe Blood Agar	BAM	•														
Cetrimide/**Pseudosel**™ Agar	BAM, USP							•	•			•				
Christensen's Urea	BAM					•			•							
Columbia Agar	USP	•		•												
Cooked Meat Medium	BAM	•														
D/E Neutralizing Broth	ISO									•						
Decarboxylase Medium	BAM					•			•							
EE Broth Mossel	USP					•*										
Fluid A	ISO									•						
Glycerol	BAM								•							
Indole Nitrite Medium/**Trypticase**™ Nitrate Broth	BAM								•							
Koser Citrate Medium	BAM								•							
Letheen Agar	AOAC									•						
Letheen Agar, Modified	BAM	•						•		•	•					•
Letheen Broth, Modified	BAM, ISO	•								•	•					•
Lysine Iron Agar	BAM					•										
MR-VP Broth	BAM					•										
MacConkey Agar	BAM, USP					•	•	•								
MacConkey Broth	USP						•									
Malonate Broth	BAM					•			•							
Malt Extract Agar	BAM															•
Mannitol Salt Agar	BAM, USP														•	
MIO Medium	BAM					•				•						
Nutrient Agar	AOAC										•					
OF Test Medium	BAM								•	•					•	
Phenylalanine Deaminase Agar	BAM					•										
Phosphate Buffer pH 7.2	USP				•											
Plate Count Agar/Standard Methods Agar	AOAC													•		
Potato Dextrose Agar	BAM, AOAC									•						•
Pseudomonas Agar F/Flo Agar	BAM								•							
Pseudomonas Agar P/Tech Agar	BAM								•							
Rappaport Vassiliadis Salmonella (RVS) Soy Broth	USP												•			

Cosmetic Testing

PRODUCTS	REFERENCE METHODS	APPLICATIONS														
		ANAEROBES	CANDIDA ALBICANS	CLOSTRIDIA	DILUENTS	ENTEROBACTERIACEAE	ESCHERICHIA COLI	GRAM-NEGATIVE SCREENING	GRAM-NEGATIVE NONFERMENTATIVE BACILLI	GRAM-POSITIVE BACTERIA	NEUTRALIZATION OF ANTIMICROBICS	PSEUDOMONAS AERUGINOSA	SALMONELLA	STANDARD PLATE COUNT	STAPHYLOCOCCUS AUREUS	YEAST & MOLD
Reinforced Clostridial Medium	USP	•		•												
Sabouraud Dextrose Agar	USP		•								•					
Sabouraud Dextrose Broth	BAM, USP		•								•			•		•
Simmons Citrate Agar	BAM					•										
Starch Agar	BAM									•					•	
TAT Broth Base	ISO										•					
Triple Sugar Iron (TSI) Agar	BAM					•		•	•							
Tryptic/**Trypticase**™ Soy Agar	BAM, ISO, USP									•	•			•	•	
Tryptic (**Trypticase**™) Soy Agar with 5% Sheep Blood	BAM	•								•						•
Tryptic (**Trypticase**™) Soy Agar with Lecithin, Poly 80 (Microbial Content Test Agar)	ISO										•		•			
Tryptic/**Trypticase**™ Soy Broth	BAM, ISO, USP				•	•	•			•	•	•	•		•	
Trypticase™ Soy Broth with 6.5% NaCl	BAM									•						
Tween™** 80	BAM								•		•					
Violet Red Bile Glucose Agar	USP					•*										
VJ (Vogel & Johnson) Agar	BAM									•					•	
XLD Agar	USP												•			
YM Agar	AOAC										•					

**Bile-tolerant gram-negative bacteria.*

***Tween is a trademark of ICI Americas, Inc.*

Environmental Sampling

PRODUCTS	CONTACT SLIDES	ISOLATOR RODAC™ PLATES	ISOLATOR SETTLING PLATES	STERILE FINGER DAB™ PLATES	STERILE PACK CONTACT PLATES	STERILE PACK RODAC™ PLATES	STERILE PACK RODAC™ SL PLATES	STERILE PACK SETTLING PLATES	STERILE PLATES (AVAILABLE ONLY AS CUSTOM PRODUCTS)
	APPLICATIONS								
CDC Anaerobe Blood Agar (**RODAC**™)									•
D/E Neutralizing Agar		•	•		•	•	•		
D/E Neutralizing Agar with Penicillinase (**RODAC**™)									•
m HPC Agar									•
Hycheck™ D/E Neutralizing Agar	•								
Hycheck™ Disinfection Control	•								
Hycheck™ *Enterobacteriaceae*	•								
Hycheck™ Plate Count Agar with TTC	•								
Hycheck™ Total Count	•								
Hycheck™ Yeast and Molds	•								
Hycheck™ Yeast and Molds with TTC	•								
R2A Agar									•
Rose Bengal Agar with Penicillinase (**RODAC**™)									•
Sabouraud Dextrose Agar					•	•			
Sabouraud Dextrose Agar with Lecithin & Polysorbate 80		•	•		•	•	•		
Trypticase™ Soy Agar			•					•	
Trypticase™ Soy Agar with Lecithin & Polysorbate 80		•	•	•	•	•	•		
Trypticase™ Soy Agar with Lecithin, Polysorbate 80 & Penicillinase					•	•	•		
Trypticase™ Soy Agar with Penicillinase								•	
Trypticase™ Soy Broth with Agar									•

Food, Dairy and Beverage Testing

PRODUCTS	REFERENCE METHODS	ANTIBIOTIC RESIDUE IN MILK	BACILLUS	CAMPYLOBACTER	CLOSTRIDIUM	COLIFORMS/E. COLI	ENTEROBACTERIACEAE	ENTEROCOCCUS	E. COLI O157	LACTOBACILLUS	LISTERIA	SALMONELLA	SHIGELLA	STAPHYLOCOCCUS	STREPTOCOCCUS	TOTAL PLATE COUNT	VIBRIO	YEAST & MOLD	YERSINIA
		APPLICATIONS																	
APT Agar	COMPF, USDA								•										
APT Broth	COMPF								•										
Alkaline Peptone Water	BAM																•		
Antibiotic Medium 1	AOAC, BAM, EP, SMD, USP	•																	
Antibiotic Medium 4	AOAC, BAM, SMD, USP	•																	
BG Sulfa Agar	CCAM, COMPF, USDA										•								
Baird-Parker Agar Base/ EY Tellurite Enrichment	AOAC, BAM, CCAM, COMPF, EP, ISO, SMD, SMWW, USDA												•						
Bile Esculin Agar	COMPF							•											
Bismuth Sulfite Agar	AOAC, BAM, CCAM, COMPF, SMD, SMWW										•								
Brain Heart Infusion	AOAC, BAM, CCAM, COMPF, ISO, SMD, SMWW, USDA			•		•							•						
Brain Heart Infusion Agar	AOAC, BAM, CCAM, COMPF, SMD, SMWW, USDA												•					•	
Brilliant Green Agar	EP, SMWW										•								
Brilliant Green Agar Modified (Edel-Kampelmacher)	ISO										•								
Brilliant Green Bile Agar	COMPF					•													
Brilliant Green Bile Broth 2%	AOAC, BAM, CCAM, COMPF, EPA, ISO, SMD, SMWW					•													
Brucella Agar	CCAM, ISO, USDA			•															
Brucella Broth	CCAM, COMPF, ISO, USDA			•															
Buffered Peptone Casein Water	ISO										•								
Buffered Peptone Water	BAM, CCAM, ISO, USDA						•				•								
Campy-Cefex Agar	USDA			•															
Campy CVA Agar	COMPF			•															
Campylobacter Agar Base/ Antimicrobic Supplement Blaser	COMPF			•															
Campylobacter Agar Base/ Antimicrobic Supplement Skirrow	ISO, SMWW			•															
Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood	SMWW			•															
BBL™ CHROMagar™ O157	AOAC-RI Approved, CCAM								•										
BBL™ CHROMagar™ Listeria	AOAC-RI Approved, BAM										•								
BBL™ CHROMagar™ Salmonella	AOAC-RI Approved											•							
BBL™ CHROMagar™ Staph aureus	AOAC-RI Approved												•						
Cooked Meat Medium	AOAC, BAM, CCAM, COMPF				•														
DNase Test Agar	COMPF												•						
DNase Test Agar with Toluidine Blue	CCAM, COMPF, SMD, BAM												•						
DRBC Agar	BAM, CCAM, COMPF, SMD																	•	
Decarboxylase Medium Base	BAM, CCAM, COMPF, ISO, SMD, SMWW					•													•
Desoxycholate Citrate Agar	EP										•	•							

Food, Dairy and Beverage Testing

PRODUCTS	REFERENCE METHODS	ANTIBIOTIC RESIDUE IN MILK	BACILLUS	CAMPYLOBACTER	CLOSTRIDIUM	COLIFORMS/E. COLI	ENTEROBACTERIACEAE	ENTEROCOCCUS	E. COLI O157	LACTOBACILLUS	LISTERIA	SALMONELLA	SHIGELLA	STAPHYLOCOCCUS	STREPTOCOCCUS	TOTAL PLATE COUNT	VIBRIO	YEAST & MOLD	YERSINIA
		APPLICATIONS																	
EC Medium/EC Broth	AOAC, BAM, CCAM, COMPF, EPA, ISO, SMD, SMWW					•													
EC Medium with MUG/ EC Broth with MUG	BAM, CCAM, EPA, SMWW					•													
EC Medium, Modified/ Novobiocin Antimicrobic Supplement	CCAM, COMPF								•										
EE Broth Mossel	COMPF, EP, ISO, JP, USP						•				•								
m Endo Agar LES	COMPF, EPA, SMD, SMWW					•													
m Endo Broth MF™	COMPF, EPA, SMD, SMWW					•													
Eosin Methylene Blue Agar, Levine	AOAC, BAM, CCAM, COMPF, SMD, USP					•	•				•	•							•
m FC Agar	AOAC, CCAM, EPA, SMWW					•													
Fluid Thioglycollate Medium	AOAC, BAM, COMPF, EP, ISO, USDA, USP				•														
Fraser Broth Base/ Fraser Broth Supplement	CCAM, COMPF, ISO, USDA									•									
GN Broth, Hajna/GN Broth	CCAM, COMPF, SMWW										•	•							
Giolitti-Cantoni Broth	ISO, COMPF												•						
M-Green Yeast and Mold Broth	COMPF																	•	
m HPC Agar	COMPF, SMWW														•				
Heart Infusion Agar	BAM, CCAM, COMPF			•												•			
Heart Infusion Broth	BAM, CCAM, COMPF, EP															•			
Hektoen Enteric Agar	AOAC, BAM, CCAM, COMPF, EP, EPA, SMD, SMWW, USP										•	•							
Kligler Iron Agar	BAM, CCAM, COMPF, ISO					•					•	•				•			•
Koser Citrate Medium	AOAC, BAM, COMPF, SMD					•													
LBS Agar	COMPF, SMD									•									
LPM Agar Base/ Listeria Selective Supplement	AOAC, BAM, CCAM, COMPF, SMD										•								
Lactobacilli MRS Agar	COMPF, ISO, SMD									•									
Lactobacilli MRS Broth	COMPF, SMD									•									
Lactose Broth	AOAC, BAM, CCAM, COMPF, EP, EPA, SMD, USP					•						•							
Lauryl Tryptose Broth/ Lauryl Sulfate Broth	AOAC, BAM, CCAM, COMPF, ISO, SMD, SMWW					•													
Lauryl Tryptose Broth with MUG/ Lauryl Sulfate Broth with MUG	AOAC, BAM, CCAM, COMPF, SMD, SMWW					•													
Listeria Enrichment Broth Base, Buffered	BAM, COMPF, SMD										•								
Liver Veal Agar	BAM, CCAM, COMPF				•														
Lysine Decarboxylase Broth	BAM, COMPF, SMD, SMWW					•	•					•	•						•
Lysine Iron Agar	AOAC, BAM, CCAM, SMD, SMWW, USDA											•	•						
M17 Agar	COMPF, ISO, SMD													•					
M Broth	AOAC, CCAM, COMPF, USDA											•							
MR-VP Broth	AOAC, BAM, CCAM, COMPF, ISO, SMD, SMWW, USDA					•	•				•	•	•						•

Food, Dairy and Beverage Testing

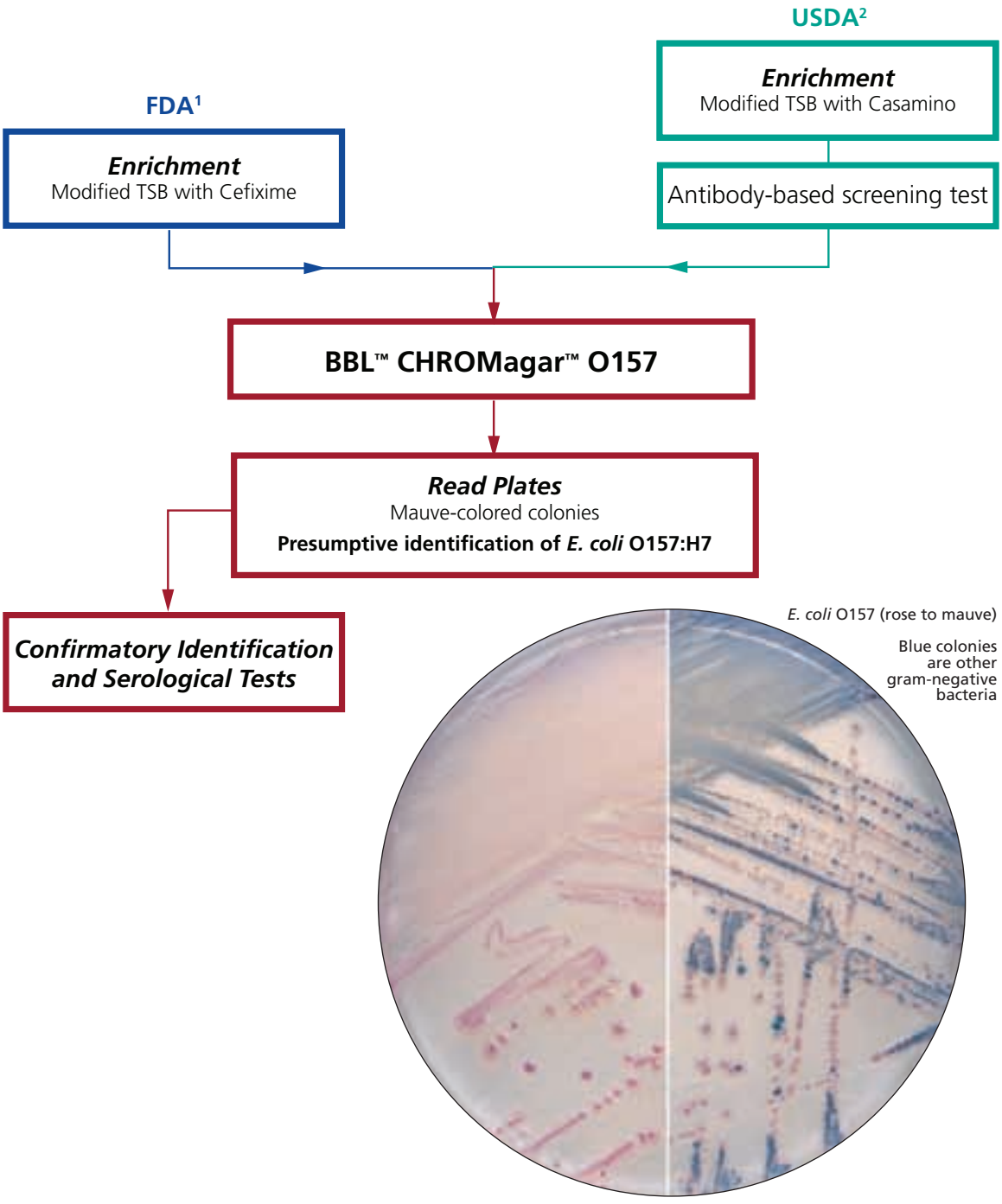
PRODUCTS	REFERENCE METHODS	ANTIBIOTIC RESIDUE IN MILK	BACILLUS	CAMPYLOBACTER	CLOSTRIDIUM	COLIFORMS/E. COLI	ENTEROBACTERIACEAE	ENTEROCOCCUS	E. COLI O157	LACTOBACILLUS	LISTERIA	SALMONELLA	SHIGELLA	STAPHYLOCOCCUS	STREPTOCOCCUS	TOTAL PLATE COUNT	VIBRIO	YEAST & MOLD	YERSINIA
		APPLICATIONS																	
MYP Agar/Egg Yolk Enrichment 50%/ Antimicrobic Vial P	AOAC, BAM, COMPF, ISO, USDA		•																
MacConkey Agar	AOAC, BAM, CCAM, COMPF, EP, JP, SMD, SMWW, USP			•		•	•					•	•						
MacConkey Broth	EP, ISO, JP, USP					•													
MacConkey Sorbitol Agar/ MacConkey II Agar with Sorbitol	BAM, CCAM, COMPF, ISO, SMWW							•											
Malonate Broth, Modified	AOAC, BAM, COMPF, SMD					•	•					•	•						
Malt Agar	AOAC, BAM																•		
Malt Extract Agar	BAM, COMPF																•		
Malt Extract Broth	BAM																•		
Mannitol Salt Agar	BAM, EP, JP, USP												•				•		
Milk Agar	ISO														•				
Moeller Decarboxylase Broth Base	COMPF, SMD, SMWW					•	•					•	•						
Motility Test Medium	BAM, CCAM, SMWW, USDA						•			•	•	•							
Mucate Broth	BAM					•													
Nutrient Agar	AOAC, BAM, CCAM, COMPF, ISO, SMWW, USDA			•		•													•
Nutrient Broth	BAM, CCAM, COMPF, SMD		•	•						•	•	•							
OF Glucose	BAM, CCAM			•			•												
OGYE Agar Base with Oxytetracycline	CCAM, COMPF, ISO																	•	
Orange Serum Agar	COMPF								•									•	
Orange Serum Broth Concentrate 10X	COMPF								•									•	
Oxford Medium Base	AOAC, BAM, CCAM, COMPF, ISO, SMD, USDA									•									
Oxford Medium, Modified/Modified Oxford Antimicrobic Supplement	AOAC, CCAM, COMPF, USDA									•									
PALCAM Medium Base/ PALCAM Antimicrobic Supplement	BAM, CCAM, COMPF, ISO, SMD									•									
Phenol Red Broth with Carbohydrates	AOAC, BAM, CCAM, COMPF		•				•					•	•						
Phenylalanine Agar	BAM						•												•
Plate Count Agar/ Standard Methods Agar	AOAC, BAM, CCAM, COMPF, EPA, ISO, SMD, SMWW, USDA														•				
Potato Dextrose Agar	AOAC, BAM, CCAM, COMPF, EP, JP, SMD, USP																	•	
Rappaport-Vassiliadis R10 Broth	ISO, USDA											•							
Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification	CCAM, ISO											•							
Rappaport Vassiliadis Salmonella (RVS) Soy Broth	CCAM, EP, ISO, JP, USDA, USP											•							
Reinforced Clostridial Medium	CCAM, EP, JP, USP				•														
Rogosa SL Agar	COMPF								•										
Rogosa SL Broth	COMPF								•										
SFP Agar Base	AOAC, BAM, COMPF, ISO				•														
SIM Medium	BAM					•	•			•	•	•							

Food, Dairy and Beverage Testing

PRODUCTS	REFERENCE METHODS	ANTIBIOTIC RESIDUE IN MILK	BACILLUS	CAMPYLOBACTER	CLOSTRIDIUM	COLIFORMS/E. COLI	ENTEROBACTERIACEAE	ENTEROCOCCUS	E. COLI O157	LACTOBACILLUS	LISTERIA	SALMONELLA	SHIGELLA	STAPHYLOCOCCUS	STREPTOCOCCUS	TOTAL PLATE COUNT	VIBRIO	YEAST & MOLD	YERSINIA
		APPLICATIONS																	
SS Agar/Salmonella Shigella Agar	COMPF, SMWW										•	•							
Sabouraud Dextrose Agar	BAM, CCAM, COMPF, EP, JP, USP																	•	
Sabouraud Dextrose Broth	BAM, CCAM, COMPF, EP, JP, USP																	•	
Selenite Cystine Broth	AOAC, BAM, CCAM, COMPF, ISO, SMD, SMWW										•	•							
Shigella Broth	BAM, CCAM, COMPF, ISO											•							
Simmons Citrate Agar	AOAC, BAM, CCAM, COMPF, ISO, SMWW					•	•				•	•							•
Sorbitol MacConkey Agar with Cefixime & Tellurite	BAM, CCAM, ISO, SMWW							•											
Staphylococcus Medium 110	BAM												•						
Sulfite Agar	AOAC, COMPF				•														
TCBS Agar	AOAC, BAM, CCAM, COMPF, ISO, SMWW																•		
m TGE Broth	COMPF															•			
TT Broth Base, Hajna	USDA										•								
Tetrathionate Broth Base	AOAC, BAM, CCAM, COMPF, EP, SMD, SMWW										•	•							
Triple Sugar Iron (TSI) Agar	AOAC, BAM, CCAM, COMPF, EP, ISO, SMD, SMWW, USDA			•		•					•	•					•		
Tryptic/**Trypticase**™ Soy Agar	AOAC, BAM, CCAM, COMPF, EP, ISO, SMD, SMWW, USDA, USP	•	•			•							•						
Tryptic (**Trypticase**™) Soy Agar with 5% Sheep Blood	BAM, CCAM, USDA		•				•			•			•	•					
Tryptic/**Trypticase**™ Soy Broth	AOAC, BAM, CCAM, COMPF, EP, ISO, SMD, SMWW, USDA, USP		•								•								
Tryptic Soy Broth without Dextrose	BAM	•																	
Tryptic Soy Broth, Modified	BAM, CCAM, ISO, USDA							•											
Tryptone Glucose Extract Agar	AOAC, COMPF															•			
Tryptone Water	ISO					•													
Tryptose Agar/Tryptose Broth	BAM, CCAM, COMPF							•		•	•	•					•		
Tryptose Phosphate Broth	BAM					•													
UVM Modified Listeria Enrichment Broth	AOAC, CCAM, COMPF, USDA									•									
Universal Preenrichment Broth	BAM, CCAM									•	•								
Urea Agar Base	CCAM, ISO, USDA																		•
VJ (Vogel & Johnson) Agar	BAM												•						
Violet Red Bile Agar	BAM, CCAM, COMPF, ISO, SMD					•													
Violet Red Bile Agar with MUG	BAM, COMPF					•													
Violet Red Bile Glucose Agar	CCAM, COMPF, EP, ISO, JP, USP					•	•												
XLD Agar	AOAC, BAM, CCAM, COMPF, EP, ISO, SMD, SMWW, USP										•	•							
XLT4 Agar Base/XLT4 Supplement	USDA										•								
YM Agar	AOAC, COMPF																	•	
Yeast Extract Glucose Chloramphenicol Agar	ISO, SMD																	•	
Yersinia Selective Agar Base/ Yersinia Antimicrobic Supplement CN	BAM, COMPF, ISO, SMD																		•

Food Testing for the Isolation and Identification of *E. coli* O157:H7 using BBL™ CHROMagar™ O157

AOAC™ – Research Institute
Performance Tested Methods℠



References
1. Feng, P. and S.D. Weagant. 2002. Diarrheagenic *Escherichia coli*, Chapter 4A, rev. Sept. 2002. *In* FDA Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. United States Department of Agriculture, Food Safety Inspection Service. 2008. Detection, isolation and identification of *Escherichia coli* O157:H7 from meat products, MLG 5.04 rev. 1/28/08. USDA/FSIS Microbiology Laboratory Guidebook, 3rd ed. U.S. Department of Agriculture, Food Safety Inspection Service, Washington, D.C.

Food Testing for the Isolation and Identification of *Listeria* using BBL™ CHROMagar™ Listeria

AOAC™ – Research Institute
Performance Tested Methods℠

USDA[2]

Pre-Enrichment
UVM Modified Listeria
Enrichment Broth

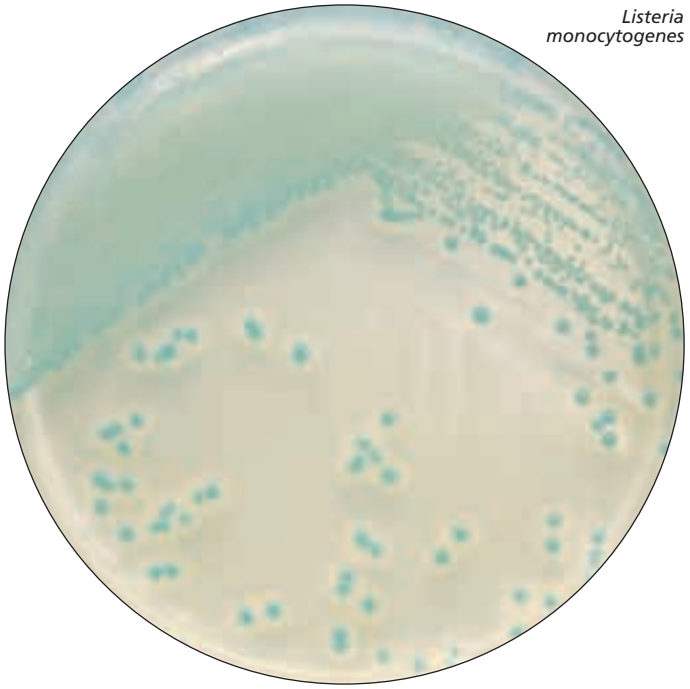
Selective Enrichment
Fraser Broth

FDA[1]

Pre-Enrichment
Buffered Listeria
Enrichment Broth

BBL™ CHROMagar™ Listeria

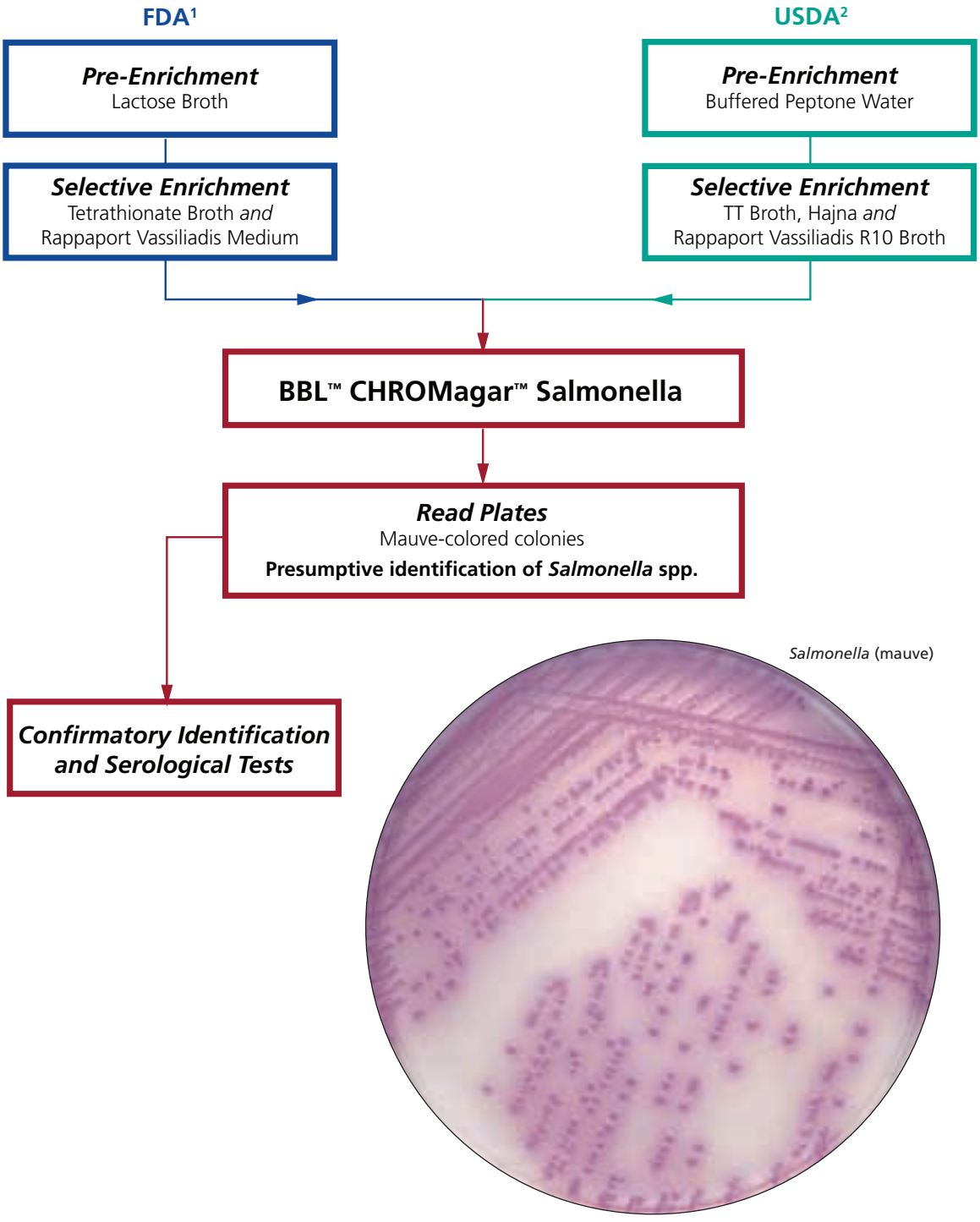
Read Plates
Blue-green colony with an opaque, white halo
Confirmatory for
Listeria monocytogenes/L. ivanovii



References
1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. Chapter 10: Detection and enumeration of *Listeria monocytogenes* in foods. AOAC International, Gaithersburg, Md.
2. U.S. Department of Agriculture, Food Safety and Inspection Services, Office of Public Health and Science. 2005. Isolation and identification of *Listeria monocytogenes* from red meat, poultry, egg and environmental samples. *In* Microbiology Laboratory Guidebook, MLG 8.04., USDA, Washington, D.C.

Food Testing for the Isolation and Identification of *Salmonella* in Meat and Meat Products using BBL™ CHROMagar™ Salmonella

AOAC™ – Research Institute
Performance Tested Methods℠



References
1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Food Testing for the Isolation, Enumeration and Identification of *Staphylococcus aureus* using BBL™ CHROMagar™ Staph aureus

AOAC™ – Research Institute
Performance Tested Methods℠

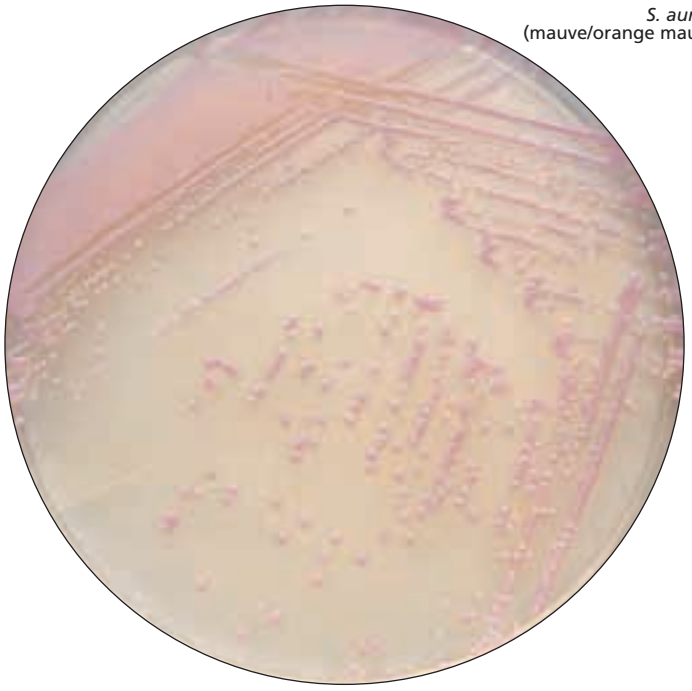
FDA[1]

Diluent
Butterfield's Phosphate Buffer

BBL™ CHROMagar™ Staph aureus

Read Plates
Mauve to orange-mauve colonies
Presumptive identification of *Staphylococcus aureus*

Confirmatory Identification Tests



Reference
1. Bennett, R.W. and G.A. Lancette. 2001. *Staphylococcus aureus*, Chapter 12, rev. Jan. 2001. *In* FDA Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Molecular Genetics Selection Guide

PRODUCTS	APPLICATIONS: GENERAL MEDIA	LB MEDIA	NZ MEDIA
2xYT	•		
Casein Digest			•
LB Agar, Lennox		•	
LB Agar, Miller		•	
LB Broth, Lennox		•	
LB Broth, Miller		•	
Luria Agar Base, Miller		•	
Luria Broth Base, Miller		•	
M9 Minimal Salts	•		
M9 Minimal Salts, 5X	•		
MacConkey Agar Base	•		
Minimal Agar Davis	•		
Minimal Broth Davis without Dextrose	•		
NZCYM Broth			•
NZYM Broth			•
NZY Broth with Thymine			•
S.O.B. Medium	•		
Select APS™ LB Broth Base		•	
Select APS™ Super Broth		•	
Select Soytone		•	
Super Broth II		•	
Terrific Broth		•	
Yeast Nitrogen Base	•		
Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate	•		
Yeast Nitrogen Base without Amino Acids	•		
YPD Agar	•		
YPD Broth	•		

Pharmaceutical Testing per USP

PRODUCTS	APPLICATIONS: ANTIBIOTIC ASSAY	ANTIMICROBIAL EFFECTIVENESS	BIOLOGICAL INDICATORS	CHAPTER <61>: MICROBIAL ENUMERATION TESTS	CHAPTER <62>: TESTS FOR SPECIFIED MICROORGANISMS	PHARMACEUTICAL WATER	STERILITY TESTING
Antibiotic Medium 1	•						
Antibiotic Medium 2	•						
Antibiotic Medium 3	•						
Antibiotic Medium 4	•						
Antibiotic Medium 5	•						
Antibiotic Medium 8	•						
Antibiotic Medium 9	•						
Antibiotic Medium 10	•						
Antibiotic Medium 11	•						
Antibiotic Medium 13	•						
Antibiotic Medium 19	•						
Buffered Sodium Chloride-Peptone Solution pH 7.0				•	•		
Cetrimide/**Pseudosel**™ Agar					•		
Columbia Agar					•		
EE Broth Mossel					•		
Fluid A							•
Fluid D							•
Fluid Thioglycollate Medium							•
m HPC Agar						•	
MacConkey Agar					•		
MacConkey Broth					•		
Mannitol Salt Agar					•		
Phosphate Buffer pH 7.2				•	•		
Plate Count Agar/Standard Methods Agar/TGY Agar						•	
Potato Dextrose Agar				•			
R2A Agar						•	
Rappaport Vassiliadis Salmonella (RVS) Soy Broth					•		
Reinforced Clostridial Medium					•		
Sabouraud Dextrose Agar		•		•	•		
Sabouraud Dextrose Broth		•		•	•		
Thioglycollate Medium, Fluid							•
Thioglycollate Medium, NIH							•
Tryptic/**Trypticase**™ Soy Agar		•	•	•	•		
Tryptic/**Trypticase**™ Soy Broth		•		•	•		•
Violet Red Bile Glucose Agar					•		
XLD Agar					•		

USP Chapter <61>: Microbial Enumeration Tests

PRODUCTS	APPLICATIONS: PREPARATION OF TEST STRAINS	PREPARATION OF SAMPLE	GROWTH PROMOTION OF MEDIA	MEMBRANE FILTRATION METHOD	MPN METHOD	POUR PLATE METHOD	SURFACE SPREAD METHOD
Buffered Sodium Chloride-Peptone Solution pH 7.0	•	•					
Phosphate Buffer pH 7.2	•	•					
Potato Dextrose Agar	•						
Sabouraud Dextrose Broth	•						
Sabouraud Dextrose Agar	•		•	•		•	•
Soybean Casein Digest Broth (Tryptic/**Trypticase**™ Soy Broth)	•	•	•		•		
Soybean Casein Digest Agar (Tryptic/**Trypticase**™ Soy Agar)	•		•	•	•	•	•

USP Chapter <62>: Tests for Specified Organisms

PRODUCTS	PREPARATION OF TEST STRAINS	GROWTH PROMOTION OF MEDIA	BUFFERS AND SOLUTIONS	PREPARATION OF TEST SAMPLES	BILE-TOLERANT GRAM-NEGATIVE BACTERIA	CANDIDA ALBICANS	CLOSTRIDIUM	ESCHERICHIA COLI	PSEUDOMONAS AERUGINOSA	SALMONELLA SPP.	STAPHYLOCOCCUS AUREUS
	APPLICATIONS										
Buffered Sodium Chloride-Peptone Solution pH 7.0	•			•							
Cetrimide Agar (**Pseudosel**™ Agar)		•							•		
Columbia Agar		•					•				
MacConkey Agar		•						•			
MacConkey Broth		•						•			
Mannitol Salt Agar		•									•
Mossel Enterobacteriaceae Enrichment Broth (EE Broth Mossel)		•			•						
Phosphate Buffer pH 7.2	•		•	•							
Potato Dextrose Agar				•							
Rappaport Vassiliadis Salmonella Enrichment Broth (RVS Soy Broth)		•								•	
Reinforced Medium for Clostridia (Reinforced Clostidial Medium)	•	•					•				
Sabouraud Dextrose Broth	•	•		•		•					
Sabouraud Dextrose Agar	•	•		•		•					
Soybean Casein Digest Broth (Tryptic/**Trypticase**™ Soy Broth)	•			•	•			•	•	•	•
Soybean Casein Digest Agar (Tryptic/**Trypticase**™ Soy Agar)	•			•							
Violet Red Bile Glucose Agar		•			•						
Xylose-Lysine Desoxycholate Agar (XLD Agar)		•								•	

Veterinary Testing

PRODUCTS	BORDETELLA	BRUCELLA	CLOSTRIDIUM	E. COLI O157	FRANCISELLA	GENERAL PURPOSE	GRAM NEGATIVE ENTERICS	LEPTOSPIRA	LISTERIA	MYCOBACTERIUM SP.	M. PARATUBERCULOSIS	MYCOPLASMA	NOCARDIA	PASTEURELLA	PSEUDOMONAS	SALMONELLA	SHIGELLA	STAPHYLOCOCCUS	STREPTOCOCCUS	VIBRIO	YEAST AND MOLD
	APPLICATIONS																				
Azide Blood Agar Base																	•	•		
Baird-Parker Agar Base/EY Tellurite Enrichment																	•			
BiGGY Agar																				•
Bismuth Sulfite Agar															•					
Bordet Gengou Agar Base	•																			
Brain Heart Infusion Broth/ Brain Heart Infusion Agar						•	•		•								•	•		•
Brilliant Green Agar															•					
Brucella Agar/Brucella Broth		•															•	•		
Casein Agar												•								
Cetrimide/**Pseudosel**™ Agar														•						
Charcoal Agar	•																			
Chocolate Agar	•																			
BBL™ **CHROMagar**™ Candida																				•
BBL™ **CHROMagar**™ Listeria									•											
BBL™ **CHROMagar**™ MRSA																	•			
BBL™ **CHROMagar**™ O157				•																
BBL™ **CHROMagar**™ Orientation							•										•	•		
BBL™ **CHROMagar**™ Salmonella															•					
BBL™ **CHROMagar**™ Staph aureus																	•			
Clostidium Difficile Selective Agar			•																	
Columbia Blood Agar Base						•														
Columbia CNA Agar																	•	•		
Cooked Meat Medium			•																	
Corn Meal Agar																				•
Cystine Heart Agar					•															
DNase Test Agar																	•			
DNase Test Agar with Methyl Green/ DNase Test Agar with Toluidine Blue																	•			
Dermatophyte Test Medium																				•
Enterococcosel™ Agar																		•		
Eosin Methylene Blue Agar, Levine							•													
Eosin Methylene Blue Agar, Modified (HHT)							•													
Eugon Agar		•			•	•			•							•		•		•
Eugon Broth/**Eugonbroth**™		•				•										•		•		•
Fletcher's Medium Base								•												
Fluid Thioglycollate Medium			•			•														
Heart Infusion Agar						•														
Hektoen Enteric Agar																•	•			
Herrold's Egg Yolk Agar with Mycobactin J and ANV											•									
Herrold's Egg Yolk Agar without Mycobactin J with ANV											•									

Veterinary Testing

PRODUCTS	BORDETELLA	BRUCELLA	CLOSTRIDIUM	E. COLI O157	FRANCISELLA	GENERAL PURPOSE	GRAM NEGATIVE ENTERICS	LEPTOSPIRA	LISTERIA	MYCOBACTERIUM SP.	M. PARATUBERCULOSIS	MYCOPLASMA	NOCARDIA	PASTEURELLA	PSEUDOMONAS	SALMONELLA	SHIGELLA	STAPHYLOCOCCUS	STREPTOCOCCUS	VIBRIO	YEAST AND MOLD
	APPLICATIONS																				
Indole Nitrite Medium/ **Trypticase**™ Nitrate Broth												•	•								
Leptospira Medium Base EMJH/ Leptospira Enrichment EMJH								•													
Liver Infusion Agar/Liver Infusion Broth		•	•															•			
Lowenstein Jensen Medium Base										•											
Lysine Iron Agar							•									•	•			•	
MIO Medium							•									•	•			•	
MR-VP Medium/MR-VP Broth							•		•							•	•			•	
MacConkey Agar							•							•	•	•	•			•	
MacConkey Sorbitol Agar/ MacConkey II Agar with Sorbitol				•			•														
Malt Agar																					•
Malt Extract Agar																					•
Mannitol Salt Agar																		•			
McClung Toabe Agar Base			•																		
Middlebrook 7H10 Agar										•											
Moeller Decarboxylase Broth Base							•									•	•				
Mycobacteria 7H11 Agar										•											
Mycoplasma Agar Base (PPLO)												•									
Mycoplasma Broth Base (PPLO)												•									
Mycoplasma Broth Base (Frey)												•									
Mycoplasma Supplement												•									
Nocardia ID QUAD												•	•								
OF Basal Media															•						
Phenol Red Carbohydrate Broths							•									•					
Phenylethyl Alcohol Agar (PEA)																		•	•		
Potato Dextrose Agar																					•
Potato Infusion Agar		•																			
PPLO Agar (Mycoplasma Agar)												•									
Pseudomonas Agar F/Flo Agar															•						
Pseudomonas Agar P/Tech Agar															•						
Purple Agar Base/Purple Broth Base							•		•							•	•			•	
Regan Lowe Charcoal Agar Base	•																				
Reinforced Clostridial Medium			•																		
SFP Agar Base			•																		
SPS Agar			•																		
SS Agar (Salmonella Shigella Agar)																•	•				
Sabouraud Dextrose Agar/ Sabouraud Dextrose Broth																					•
Schaedler Agar			•																		
Selenite Cystine Broth																•	•				

Veterinary Testing

PRODUCTS	BORDETELLA	BRUCELLA	CLOSTRIDIUM	E. COLI O157	FRANCISELLA	GENERAL PURPOSE	GRAM NEGATIVE ENTERICS	LEPTOSPIRA	LISTERIA	MYCOBACTERIUM SP.	M. PARATUBERCULOSIS	MYCOPLASMA	NOCARDIA	PASTEURELLA	PSEUDOMONAS	SALMONELLA	SHIGELLA	STAPHYLOCOCCUS	STREPTOCOCCUS	VIBRIO	YEAST AND MOLD
	APPLICATIONS																				
Simmons Citrate Agar							•														
Staphylococcus Medium 110																	•				
TCBS Agar																			•		
Tetrathionate Broth Base															•						
Triple Sugar Iron (TSI) Agar							•		•												
Tryptic/**Trypticase**™ Soy Agar/ Tryptic/**Trypticase**™ Soy Broth						•			•												
Tryptose Agar/Tryptose Broth		•																			
Tryptose Phosphate Broth						•															
Tyrosine Agar													•								
Urea Agar Base							•														
VJ (Vogel & Johnson) Agar																	•				
XLD Agar															•	•					
Xanthine Agar													•								
YM Agar/YM Broth																					•

Water/Wastewater Testing

PRODUCTS	REFERENCE METHODS	APPLICATIONS																
		AEROMONAS	CAMPYLOBACTER	COLIFORMS - TOTAL	COLIPHAGES	E. COLI	E. COLI O157	FECAL COLIFORMS	FECAL STREPTOCOCCUS	FUNGI	LEGIONELLA	PSEUDOMONAS	SALMONELLA	SHIGELLA	STANDARD PLATE COUNT	STAPHYLOCOCCUS	STRESSED ORGANISMS	VIBRIO
A-1 Medium	COMPF, EPA, SMWW							•										
Alkaline Peptone Water	SMWW	•																
Azide Dextrose Broth	EPA, SMWW								•									
BCYE Agar	SMWW									•								
Baird-Parker Agar Base with EY Tellurite Enrichment	AOAC, BAM, CCAM, COMPF, EP, ISO, SMD, SMWW, USDA														•			
Beef Extract	AOAC, COMPF, EPA, SMWW				•													
Bismuth Sulfite Agar	AOAC, BAM, CCAM, COMPF, SMD, SMWW											•						
Brain Heart Infusion	AOAC, BAM, COMPF, EPA, SMD, SMWW, USDA								•							•		
Brain Heart Infusion Agar	AOAC, BAM, CCAM, COMPF, EPA, SMD, SMWW, USDA								•									
Brilliant Green Agar	EP, SMWW											•						
Brilliant Green Agar Modified (Edel-Kempelmacher)	ISO											•						
Brilliant Green Bile Broth 2%	AOAC, BAM, CCAM, COMPF, EPA, ISO, SMD, SMWW	•		•														
Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood	SMWW		•															
Campylobacter Agar Base/ Antimicrobic Supplement Skirrow	ISO, SMWW		•															
Cetrimide Agar	AOAC, BAM, CCAM, EP, JP, USP										•							
Cooke Rose Bengal Agar/ Antimicrobic Vial A	SMWW									•								
Czapek Solution Agar	SMWW									•								
Decarboxylase Medium Base	BAM, CCAM, COMPF, ISO, SMD, SMWW					•												
m E Agar	EPA, SMWW								•									
EC Medium	AOAC, BAM, CCAM, COMPF, EPA, ISO, SMD, SMWW			•		•												
EC Medium with MUG	BAM, CCAM, EPA, SMWW					•												
m EI Agar	EPA								•									
m Endo Agar LES	COMPF, SMD, SMWW			•			•											
m Endo Broth MF™	COMPF, SMD, SMWW			•														
Enterococcosel™ Agar (Bile Esculin Azide Agar)	ISO								•									
m Enterococcus Agar	ISO, SMWW								•									
Eosin Methylene Blue Agar, Levine	AOAC, BAM, CCAM, COMPF, SMD, USP					•	•					•	•	•				•
Esculin Iron Agar (EIA Substrate)	EPA, SMWW								•									
m FC Agar	AOAC, CCAM, EPA, SMWW							•										
m FC Broth Base	EPA, SMWW							•										
GN Broth, Hajna/GN Broth	CCAM, COMPF, SMWW											•	•					

Water/Wastewater Testing

PRODUCTS	REFERENCE METHODS	APPLICATIONS																
		AEROMONAS	CAMPYLOBACTER	COLIFORMS - TOTAL	COLIPHAGES	E. COLI	E. COLI O157	FECAL COLIFORMS	FECAL STREPTOCOCCUS	FUNGI	LEGIONELLA	PSEUDOMONAS	SALMONELLA	SHIGELLA	STANDARD PLATE COUNT	STAPHYLOCOCCUS	STRESSED ORGANISMS	VIBRIO
m HPC Agar	SMWW, COMPF													•				
Lauryl Tryptose Broth/ Lauryl Sulfate Broth	AOAC, BAM, CCAM, COMPF, ISO, SMD, SMWW	•		•		•		•										
Lauryl Tryptose Broth with MUG/ Lauryl Sulfate Broth with MUG	AOAC, BAM, COMPF, SMD, SMWW					•												
Lysine Iron Agar	AOAC, BAM, CCAM, COMPF, SMD, SMWW, USDA	•				•						•	•	•			•	•
MI Agar	EPA			•		•												
MR-VP Broth	AOAC, BAM, CCAM, COMPF, ISO, SMD, SMWW, USDA	•				•							•	•				•
MacConkey Agar	AOAC, BAM, CCAM, COMPF, EP, JP, SMD, SMWW, USP	•	•			•	•					•	•	•				•
MacConkey Sorbitol Agar/ MacConkey II Agar with Sorbitol	BAM, CCAM, COMPF, ISO, SMWW						•											
Moeller Decarboxylase Broth Base	COMPF, SMD, SMWW	•				•						•	•	•				
Motility Test Medium	BAM, CCAM, COMPF, SMWW, USDA			•								•	•	•				•
Nutrient Agar	AOAC, BAM, CCAM, COMPF, SMWW, USDA			•		•												
Nutrient Agar with MUG	EPA, SMWW					•												
M-PA-C Agar	CCAM, SMWW											•						
Plate Count Agar/ Standard Methods Agar	AOAC, BAM, CCAM, COMPF, EPA, ISO, SMD, SMWW, USDA														•			
Presence-Absence Broth	CCAM, EPA, SMWW			•														
R2A Agar	COMPF, EPA, SMWW																•	
Rose Bengal Agar Base/Rose Bengal Antimicrobic Supplement C	SMWW									•								
Sabouraud Dextrose Agar, Emmons	SMWW									•								
Selenite F Broth	SMWW												•					
Selenite Cystine Broth	AOAC, BAM, CCAM, COMPF, ISO, SMD, SMWW												•	•				
Simmons Citrate Agar	AOAC, BAM, CCAM, COMPF, ISO, SMWW					•												
Sorbitol MacConkey Agar with Cefixime & Tellurite	BAM, CCAM, ISO, SMWW						•											
m Staphylococcus Broth	SMWW															•		
TCBS Agar	AOAC, BAM, CCAM, COMPF, ISO, SMWW																	•
m TEC Agar	SMWW					•												
m TEC Agar, Modified	EPA					•												
Tetrathionate Broth Base	AOAC, BAM, CCAM, COMPF, EP, SMD, SMWW												•					
Triple Sugar Iron (TSI) Agar	AOAC, BAM, CCAM, COMPF, EP, ISO, SMD, SMWW, USDA	•		•		•							•	•				•

Water/Wastewater Testing

PRODUCTS	REFERENCE METHODS	APPLICATIONS: AEROMONAS	CAMPYLOBACTER	COLIFORMS - TOTAL	COLIPHAGES	E. COLI	E. COLI O157	FECAL COLIFORMS	FECAL STREPTOCOCCUS	FUNGI	LEGIONELLA	PSEUDOMONAS	SALMONELLA	SHIGELLA	STANDARD PLATE COUNT	STAPHYLOCOCCUS	STRESSED ORGANISMS	VIBRIO
Tryptic/**Trypticase**™ Soy Agar	AOAC, BAM, CCAM, COMPF, EP, EPA, ISO, SMD, SMWW, USDA, USP				•	•						•		•			•	
Tryptic/**Trypticase**™ Soy Broth	AOAC, BAM, CCAM, COMPF, EP, EPA, ISO, JP, SMD, SMWW, USDA, USP	•			•	•								•				
Tryptone Water (1%)	ISO					•												
TTC Solution, 1%	EPA, SMWW								•									
XLD Agar	AOAC, BAM, CCAM, COMPF, EP, ISO, SMD, SMWW, USP												•	•				

Water Testing for the Enumeration of *Enterococcus* in Recreational Water using BBL™ mEI Agar[1]

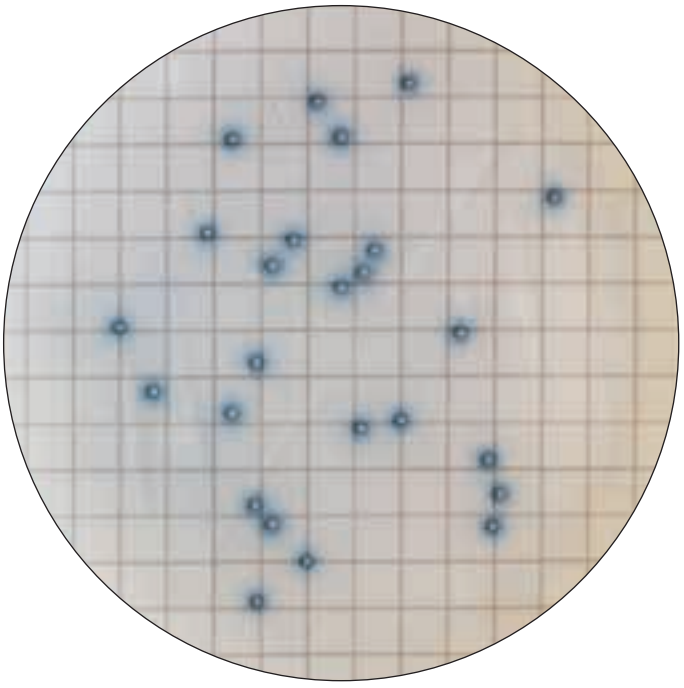
Collect water sample and run through membrane filter

↓

Roll membrane filter onto **BBL™ mEI Agar**

↓

Colonies with a blue halo, regardless of color, are ***Enterococcus***. No additional testing required.



Reference

1. U.S. Environmental Protection Agency (USEPA). 2002. Method 1600: Enterococci in water by membrane filtration using membrane-*Enterococcus* Indoxyl-β-D-Glucoside Agar (mEI). EPA-821-R-02-022. Office of Water, Washington, D.C.

Water Testing for the Enumeration of *E. coli* in Recreational Water using BBL™ Modified mTEC Agar[1]

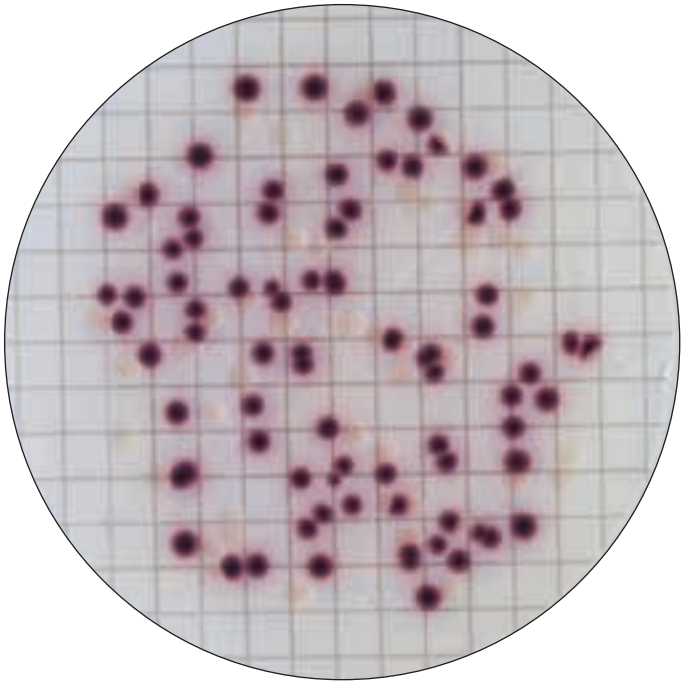
Collect water sample and run through membrane filter

↓

Roll membrane filter onto
BBL™ Modified mTEC Agar

↓

Colonies of ***E. coli*** are red or magenta in color.
No additional testing required.



Reference
1. U.S. Environmental Protection Agency (USEPA). 2002. Method 1603: *Escherichia coli* (*E.coli*) in water by membrane filtration using Modified membrane-Thermotolerant *Escherichia coli* agar (Modified mTEC). EPA-821-R-02-023. Office of Water (4303T), Washington, D.C.

Water Testing for the Enumeration of Total Coliforms and *E. coli* in Drinking Water using BBL™ MI Agar[1]

Collect water sample and run through membrane filter

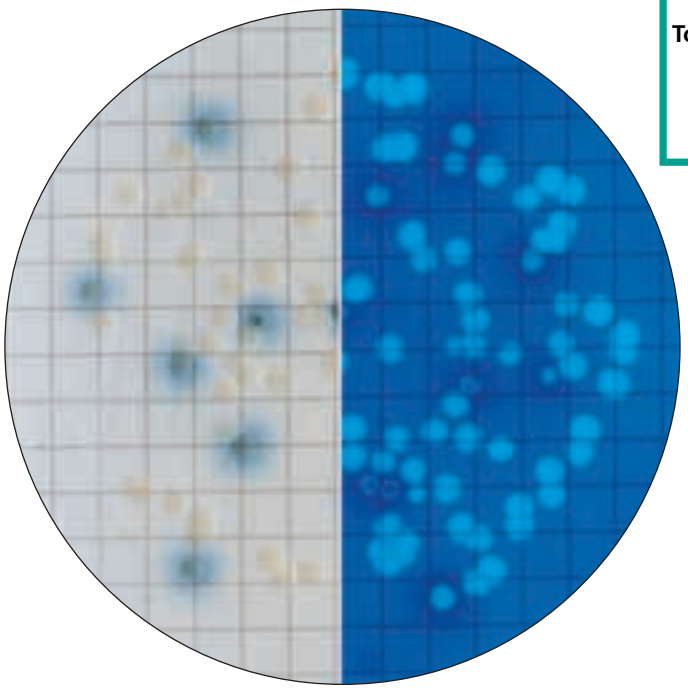
Roll membrane filter onto
BBL™ MI Agar

E. coli
Count all blue colonies under normal/ambient light to obtain ***E.coli*** count. No additional testing required.

Expose plate to UV light (366 nm)

Total Coliforms
Count all fluorescent colonies + any blue, non-fluorescent colonies (*E. coli*) to obtain **Total Coliform** count. No additional testing required.

Fluorescent colonies may be blue/green (*E. coli*), blue/white (total coliforms other than *E. coli*), or blue/green with fluorescent edges (also *E. coli*).



Reference
1. U.S. Environmental Protection Agency (USEPA). 2002. Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI Medium). EPA 821-R-02-024. Office of Water (4303T), Washington, D.C.

Culture Media — Antimicrobial Susceptibility Testing

PRODUCTS
Anaerobe MIC Broth
Brain Heart Infusion
Brucella Agar (supplemented with lysed sheep blood, hemin and vitamin K1)
Enriched Thioglycollate Medium (supplemented with sodium bicarbonate or a marble chip)
Enterococcosel™ Agar with Vancomycin, 8 µg/mL
GC II Agar with **IsoVitaleX**™ Enrichment
Haemophilus Test Medium Agar
Mueller Hinton Agar
Mueller Hinton Agar with 5% Sheep Blood
Mueller Hinton II Agar
Mueller Hinton Broth
Mueller Hinton II Broth
Mueller Hinton II Broth with 2% Sodium Chloride
Oxacillin Screen Agar
Tryptic/**Trypticase**™ Soy Broth
Vancomycin Screen Agar
Wilkins Chalgren Agar

Culture Media — General Purpose

PRODUCTS		
Brain Heart Infusion	Heart Infusion Broth	Tryptic Soy Blood Agar Base No. 2
Brain Heart Infusion Agar	Infusion Agar	Tryptic/**Trypticase**™ Soy Agar
Brain Heart Infusion Agar, Modified	Infusion Broth	**Trypticase**™ Soy Agar with 5% Sheep Blood
Brain Heart Infusion Broth, Modified	Nutrient Agar	**Trypticase**™ Soy Agar with Lecithin and Polysorbate 80
Brain Heart Infusion without Dextrose	Nutrient Agar 1.5%	**Trypticase**™ Soy Broth with 10% NaCl
Columbia Agar Base	Nutrient Broth	**Trypticase**™ Soy Broth with 5% Fildes
Columbia Blood Agar Base	Schaedler Agar	**Trypticase**™ Soy Broth with Lecithin and Polysorbate 80
Columbia Blood Agar Base EH	Schaedler Agar with Vitamin K1 and 5% Sheep Blood	**Trypticase**™ Soy Broth without Dextrose
Columbia Agar with 5% Sheep Blood	Schaedler KV Agar with 5% Sheep Blood	Tryptose Agar
Columbia Anaerobe 5% Sheep Blood Agar	Thioglycollate Medium without Indicator	Tryptose Blood Agar Base
Columbia CNA Agar	Thioglycollate Medium, Brewer Modified	Tryptose Broth
Columbia CNA Agar with 5% Sheep Blood	Thioglycollate Medium (Fluid), Enriched	Tryptose Phosphate Broth
Columbia PNA Agar with 5% Sheep Blood	Thioglycollate Medium (Fluid), Enriched w/Vitamin K1 & Hemin	Veal Infusion Agar
Columbia Broth	Tryptic Soy Blood Agar Base EH	Veal Infusion Broth
GC Medium Base		
Heart Infusion Agar		

Culture Media — Supplements/Selective Agents

CULTURE MEDIUM	SUPPLEMENT	AGENT	CONCENTRATION PER VIAL
Baird-Parker Agar Base	EY Tellurite Enrichment	Egg Yolk emulsion containing Potassium Tellurite	
Campylobacter Agar Base	Campylobacter Antimicrobic Supplement Skirrow	Vancomycin - Polymyxin B - Trimethoprim	5 mg/1,250 units/2.5 mg per 5 mL vial
Campylobacter Agar Base	Campylobacter Antimicrobic Supplement Blaser	Vancomycin - Polymyxin B - Trimethoprim - Cephalothin - Amphotericin B	5 mg/1,250 units/2.5 mg/ 7.5 mg/1 mg per 5 mL vial
Cooke Rose Bengal Agar	Antimicrobic Vial A	Chlortetracycline	25 mg
Demi-Fraser Broth Base	Fraser Broth Supplement	Ferric Ammonium Citrate	0.5 g
Dubos Broth Base	Dubos Medium Albumin	Solution of Albumin Fraction V (from bovine plasma) and Dextrose in normal saline	5% solution of albumin fraction V/ 7.5% dextrose in normal saline
Dubos Oleic Agar Base	Dubos Oleic Albumin Complex	Solution of alkalinized Oleic Acid in a solution of Albumin Fraction V	0.05% solution of oleic acid in a 5% solution of albumin fraction V in normal saline
EC Medium, Modified	Novobiocin Antimicrobic Supplement	Novobiocin	20 mg
m FC Agar/m FC Broth Base	Rosolic Acid	Rosolic Acid	1.0 g
Fraser Broth Base	Fraser Broth Supplement	Ferric Ammonium Citrate	0.5 g
GC Medium Base	VCA Inhibitor	Vancomycin - Colistin - Anisomycin	400 µg/750 µg/2.0 mg
GC Medium Base	VCAT Inhibitor	Vancomycin - Colistin - Anisomycin - Trimethoprim Lactate	400 µg/750 µg/2.0 mg/5 µg
GC Medium Base	VCN Inhibitor	Vancomycin - Colistin - Nystatin	300 µg/750 µg/1,250 units
GC Medium Base	VCNT Inhibitor	Vancomycin - Colistin - Nystatin - Trimethoprim Lactate	300 µg/750 µg/1,250 units/5 µg
GC Medium Base	Supplement B	Sterile yeast concentrate containing V and X factors	
GC Medium Base	Supplement VX	Sterile concentrate of essential growth factors	
GC Medium Base	**IsoVitaleX**™ Enrichment	Chemically defined supplement containing V factor	
GC Medium Base	Hemoglobin Solution 2%	Hemoglobin provides the X factor	2%
KF Streptococcus Agar	TTC Solution 1%	2,3,5-Triphenyl Tetrazolium Chloride	1%
LPM Agar Base	Listeria Selective Supplement	Moxalactam	20 mg
Legionella Agar Base	Legionella Agar Enrichment	L-Cysteine HCl-Ferric Pyrophosphate	0.35 g/0.14 g per 5 mL
Leptospira Medium Base EMJH	Leptospira Enrichment EMJH	Solution of Albumin, Polysorbate 80 and additional growth factors for *Leptospira*	
MYP Agar	Antimicrobic Vial P	Polymyxin B	30,000 units
McClung Toabe Agar Base	Egg Yolk Enrichment 50%	Sterile concentrated egg yolk emulsion	50% egg yolk/50% saline
Middlebrook 7H9 Broth	Middlebrook ADC Enrichment	Albumin Fraction V, Bovine - Dextrose - Catalase (Beef)	5 g/2 g/0.003 g per 100 mL
Middlebrook 7H10 Agar	Middlebrook OADC Enrichment	Oleic Acid - Albumin Fraction V, Bovine - Dextrose - Catalase (beef) - Sodium Chloride	0.05g/ 5 g/2 g/0.0004 g/0.85 g per 100 mL
Mitis Salivarius Agar	Tellurite Solution 1%	Potassium Tellurite Solution	1%
Oxford Medium Base	Modified Oxford Antimicrobic Supplement	Moxalactam - Colistin Sulfate	20 mg/10 mg
PALCAM Medium Base	PALCAM Antimicrobic Supplement	Ceftozidime	40 mg
PPLO Agar/PPLO Broth without CV	Mycoplasma Supplement	Horse Serum, Dessicated - Yeast Extract	1.6 g/0.01 g
PPLO Agar/PPLO Broth without CV	Mycoplasma Enrichment without Penicillin	Horse Serum - Yeast Extract - Thallium Acetate	20 mL/10 mL/50 mg
Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification	Novobiocin Antimicrobic Supplement	Novobiocin	20 mg
Rose Bengal Agar Base	Rose Bengal Antimicrobic Supplement C	Chloramphenicol	0.05 g
SFP Agar Base	Egg Yolk Enrichment 50% Antimicrobic Vial K Antimicrobic Vial P	Sterile Concentrated Egg Yolk Emulsion Kanamycin Polymyxin B	50% egg yolk/50% saline 25,000 µg 30,000 units
Spirit Blue Agar	Lipase Reagent	Lipid suspension containing a mixture of Tributyrin and Polysorbate 80	
Tellurite Glycine Agar	Tellurite Solution 1%	Potassium Tellurite Solution	1%
Tinsdale Agar	Tinsdale Enrichment Desiccated	Contains Bovine Serum, Horse Serum, L-cystine, Sodium Thiosulfate and Potassium Tellurite	
XLT4 Agar Base	XLT4 Supplement	Sodium 7 - ethyl - 2 - methyl - 4 - undecyl sulfate (Tergitol™ 4)	
Yersinia Selective Agar Base (CIN)	Yersinia Antimicrobic Supplement CN	Cefsulodin - Novobiocin	4 mg/2.5 mg

Peptones and Hydrolysates

PRODUCT NAME	100 G	500 G	454 G	2 KG	5 LB	10 KG	25 LB	25 KG	50 KG
Acidicase™ Peptone		211843							
Beef Extract, Paste		212610							
Beef Extract, Powder		212303							
Beef Extract, Desiccated, **Bacto**™		211520							
Beef Heart for Infusion		213210							
Biosate™ Peptone			211862				294312		
Brain Heart Infusion, Porcine, **Bacto**™						256110			
Casamino Acids, **Bacto**™		223050		223020		223030			
Casamino Acids, Technical, **Bacto**™		223120				223110			
Casamino Acids, Vitamin Assay	228820	228830							
Casitone, **Bacto**™		225930				225910			
Gelysate™ Peptone			211870						
Malt Extract, **Bacto**™		218630				218610			
Neopeptone, **Bacto**™		211681				211680			
Peptone, **Bacto**™		211677		211820		211830			
Phytone™ Peptone			211906		298147	292450			
Phytone™ UF (Select)		210931				210936			
Polypeptone™ Peptone			211910			297108			
Proteose Peptone, **Bacto**™		211684				212010			212030
Proteose Peptone, **BiTek**™						253310			
Proteose Peptone No. 2, **Bacto**™		212120				212110			
Proteose Peptone No. 3, **Bacto**™		211693		212220		212230			211692
Proteose Peptone No. 3, **BiTek**™								253720	
Proteose Peptone No. 4, **Bacto**™						211715			
Select Soytone		212488				212489			
Soytone, **Bacto**™		243620				243610			
TC Lactalbumin Hydrolysate, **Bacto**™		259962				259961			
TC Yeastolate, **Bacto**™	255772					255771			
TC Yeastolate, UF		292804				292805			
Trypticase™ Peptone			211921		211922		211923		299031
Tryptone, **Bacto**™		211705		211699		211701			
Tryptone, **BiTek**™						251420			
Tryptose, **Bacto**™		211713				211709			
Yeast Extract			211929		211930		211931		
Yeast Extract, **Bacto**™		212750		212720		212730			212710
Yeast Extract, LD		210933				210941			
Yeast Extract, Technical, **Bacto**™		288620				288610			
Yeast Extract, UF		210929				210934			

Peptones by Category

MEAT PEPTONES AND MEDIA	
Beef Extract, Paste	**Polypeptone**™ Peptone
Beef Extract, Powder	**Bacto**™ Proteose Peptone
Bacto™ Beef Extract, Desiccated	**BiTek**™ Proteose Peptone
Beef Heart for Infusion	**Bacto**™ Proteose Peptone No. 2
Bacto™ Brain Heart Infusion	**Bacto**™ Proteose Peptone No. 3
Bacto™ Brain Heart Infusion, Porcine	**BiTek** Proteose Peptone No. 3
Bacto™ Neopeptone Peptone	**Bacto**™ Proteose Peptone No. 4
Bacto™ Peptone	**Bacto**™ Tryptose
Gelysate™ Peptone	
CASEIN PEPTONES	
Bacto™ Casamino Acids	**Trypticase**™ Peptone
Bacto™ Casitone	**Bacto**™ TC Lactalbumin Hydrolysate
Acidicase™ Peptone	**Bacto**™ Tryptone
Biosate™ Peptone	**BiTek** Tryptone
SOY PEPTONES	
Phytone™ Peptone	Select Soytone
Phytone™ UF (Select)	**Bacto**™ Soytone
YEAST EXTRACTS	
Bacto™ TC Yeastolate	Yeast Extract
TC Yeastolate, UF	Yeast Extract, UF
Bacto™ Yeast Extract	Yeast Extract, LD
Bacto™ Yeast Extract, Technical	
ALTERNATIVE PROTEIN SOURCE (APS)/NON-ANIMAL ORIGIN PRODUCTS	
Bacto™ Malt Extract	**Bacto**™ Yeast Extract, Technical
Phytone™ Peptone	Yeast Extract
Phytone™ UF (Select)	Yeast Extract, UF
Select Soytone	Yeast Extract, LD
Bacto™ TC Yeastolate	**Select APS**™ LB Broth
TC Yeastolate, UF	**Select APS**™ Super Broth
Bacto™ Yeast Extract	

Typical Analyses - Peptones and Hydrolysates

Product Name	Total Nitrogen (%)	Amino Nitrogen (%)	AN/TN	Ash (%)	Loss on Drying (%)	NaCl (%)	pH (2% Solution)	Calcium (ug/g)	Magnesium (ug/g)	Potassium (ug/g)	Sodium (ug/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% Free)	Alanine (% Total)	Arginine (% Free)	Arginine (% Total)	Asparagine (% Free)	Aspartic Acid (% Free)	Aspartic Acid (% Total)
Acidicase™ Peptone	8.5	6.2	0.73	36.8	5.3	32.3	6.8	229	36	1164	140900	16.99	0.25	1.42	1.6	2.1	1.3	1.9	0.0	3.4	3.9
Beef Extract, Desiccated, **Bacto**™	13.9	2.0	0.14	7.7	1.8	1.7	6.9	53	92	31423	21645	1.62	0.70	0.43	1.1	7.1	1.3	4.2	0.1	0.3	2.4
Beef Extract, Powder	12.4	2.3	0.19	9.3	3.5	0.3	6.9	264	285	28793	18510	*	0.53	3.22	1.8	4.0	2.8	2.8	0.6	0.6	5.5
Biosate™ Peptone	13.4	6.0	0.45	7.7	6.6	0.3	7.1	258	398	21320	17100	0.07	0.43	3.19	2.4	4.2	2.1	2.9	0.9	0.9	5.9
Brain Heart Infusion, **Bacto**™	10.2	3.9	0.38	22.9	1.5	3.2	7.3	103	394	19810	72870	9.05	0.25	0.22	1.3	6.5	1.1	3.2	0.4	0.7	3.9
Brain Heart Infusion, Porcine, **Bacto**™	9.2	4.0	0.43	22.4	1.8	2.2	7.4	211	374	19020	80930	8.77	0.27	0.25	1.4	6.6	0.9	3.5	0.4	0.9	3.8
Casamino Acids, **Bacto**™	10.8	9.4	0.87	18.3	4.8	12.1	6.4	59	2	4098	88090	6.74	0.55	2.56	3.0	3.0	2.4	2.5	0.0	5.0	5.0
Casamino Acids, **BiTek**™	8.3	5.7	0.69	33.7	4.0	14.3	6.9	110	70	1740	144360	18.25	0.26	1.53	2.1	4.4	1.1	1.7	0.0	3.6	3.2
Casitone, **Bacto**™	13.5	5.0	0.37	6.4	2.0	0.0	7.0	111	213	3480	34090	0.10	0.40	2.48	0.9	3.4	2.6	2.8	0.5	0.2	5.5
Gelysate™ Peptone	17.0	2.9	0.17	3.8	4.9	0.2	6.9	650	150	646	11090	*	1.66	0.18	0.8	8.8	3.1	6.3	0.1	0.1	4.7
Malt Extract, **Bacto**™	0.3	0.3	0.97	0.3	3.1	0.2	5.2	111	130	603	713	0.07	0.07	0.08	0.1	0.1	*	0.1	*	*	0.1
Neopeptone, **Bacto**™	13.6	3.2	0.20	6.9	4.0	1.4	7.4	38	8	8945	36313	0.48	0.45	2.59	0.5	4.3	0.5	2.6	0.2	0.3	4.2
Peptone, **Bacto**™	15.4	3.5	0.20	3.8	2.7	1.7	7.1	18	1	2542	18440	0.90	0.32	0.40	1.2	9.2	2.8	5.8	0.3	0.3	5.0
Phytone™ Peptone	9.0	2.4	0.27	12.4	1.5	4.0	7.1	1001	2435	31547	34037	0.76	0.67	0.64	0.3	2.6	0.6	2.1	0.1	0.3	3.9
Phytone™ Peptone UF	9.4	2.6	0.28	12.5	4.9	4.0	7.0	900	1700	21200	36100	0.76	0.58	0.71	0.3	3.1	0.8	2.4	0.2	0.2	4.7
Polypeptone™ Peptone	13.1	5.2	0.40	9.7	4.9	2.7	7.3	271	342	7340	44257	1.00	0.40	3.40	1.2	4.1	2.4	3.3	0.4	0.4	6.1
Proteose Peptone, **Bacto**™	14.3	2.8	0.20	7.8	3.0	4.9	6.7	120	261	12780	23110	2.65	0.19	0.64	0.5	6.0	0.4	4.7	0.1	0.4	5.3
Proteose Peptone, **BiTek**™	13.1	3.1	0.24	13.1	4.8	10.3	6.8	130	680	7390	49930	4.93	1.01	0.94	0.8	7.0	0.4	4.4	0.1	0.6	3.9
Proteose Peptone No. 2, **Bacto**™	12.9	5.0	0.39	12.1	3.5	7.1	7.3	151	98	13313	52717	3.86	0.38	1.88	1.6	5.2	1.4	4.1	0.5	1.1	5.5
Proteose Peptone No. 3, **Bacto**™	13.4	3.7	0.28	10.5	2.3	6.6	7.4	132	67	13160	38113	2.54	0.37	1.51	0.9	5.2	0.8	4.3	0.3	0.6	5.1
Proteose Peptone No. 4, **Bacto**™	14.3	2.7	0.19	7.8	3.3	3.9	7.0	169	378	11243	35280	2.63	0.34	0.72	0.5	6.5	0.4	4.6	0.1	0.3	4.4
Select Soytone	9.2	3.7	0.40	10.7	3.5	0.0	7.0	250	1749	29787	31087	0.07	2.65	1.03	0.5	3.6	0.4	2.1	0.4	0.2	6.2
Soytone, **Bacto**™	9.4	3.1	0.33	12.0	4.6	0.2	7.2	550	1610	22200	34040	0.17	2.33	0.82	0.4	2.5	2.1	2.8	0.3	0.2	5.5
TC Lactalbumin Hydrolysate	13.0	6.3	48.30	7.2	4.6	0.3	7.0	1620	340	17200	14800	0.8	1.2	4.1	2.3	4.7	2.2	2.5	0.9	0.9	6.5
TC Yeastolate UF	10.6	6.5	0.61	13.3	2.1	1.0	7.0	247	267	60940	3716	0.52	0.89	2.46	5.5	5.7	1.9	3.2	1.3	2.1	6.4
TC Yeastolate, **Bacto**™	10.7	6.0	0.56	11.7	2.2	0.6	7.0	20	250	50850	8190	0.30	0.49	2.63	4.6	4.6	1.7	2.4	1.2	1.8	4.8
Trypticase™ Peptone	14.2	5.2	0.37	5.7	4.0	0.1	7.2	295	110	588	26600	0.09	0.18	2.54	0.9	5.7	2.3	4.8	0.5	0.2	7.7
Tryptone, **Bacto**™	13.3	5.3	0.40	6.6	2.3	0.0	7.3	256	195	3257	33910	0.06	0.33	2.58	1.0	3.2	3.1	2.7	0.6	0.4	5.2
Tryptone, **BiTek**™	13.1	5.6	0.43	5.8	5.0	0.0	7.1	180	100	620	26970	0.35	0.22	2.25	0.6	5.0	3.8	2.6	0.5	0.1	3.9
Tryptose, **Bacto**™	13.3	4.5	0.34	8.8	3.2	3.2	7.3	191	110	9292	37740	1.61	0.23	2.05	1.2	4.3	1.9	3.5	0.4	0.5	5.1
Yeast Extract	11.4	6.9	0.60	13.1	1.0	0.2	7.0	230	799	58013	1003	0.07	0.65	3.73	5.7	6.2	2.0	3.0	1.0	2.2	5.9
Yeast Extract, **Bacto**™	10.9	6.0	0.55	11.2	3.1	0.1	6.7	130	750	31950	14900	0.38	0.09	3.27	4.4	5.6	1.4	2.6	1.0	1.6	5.3
Yeast Extract, LD	8.1	6.1	0.75	17.5	0.3	0.1	7.0	254	649	55700	1683	0.12	0.96	2.11	4.7	5.1	1.8	2.6	1.2	1.9	5.2
Yeast Extract, UF	10.7	6.0	0.56	18.2	0.7	0.0	7.0	191	558	59240	1244	0.13	1.02	2.70	4.8	5.4	1.5	2.6	1.2	1.7	5.4

*Below level of detection

**Partially destroyed during hydrolysis

Free Amino Acids/Total Amino Acids

Cystine (% Free)	Glutamic Acid (% Free)	Glutamic Acid (% Total)	Glutamine (% Free)	Glycine (% Free)	Glycine (% Total)	Histidine (% Free)	Histidine (% Total)	Isoleucine (% Free)	Isoleucine (% Total)	Leucine (% Free)	Leucine (% Total)	Lysine (% Free)	Lysine (% Total)	Methionine (% Free)	Methionine (% Total)	Phenylalanine (% Free)	Phenylalanine (% Total)	Proline (% Free)	Proline (% Total)	Serine (% Free)	Serine (% Total)**	Threonine (% Free)	Threonine (% Total)	Tryptophan (% Free)	Tyrosine (% Free)	Tyrosine (% Total)	Valine (% Free)	Valine (% Total)
0.8	8.3	11.6	0.0	0.8	1.0	0.8	1.6	1.6	4.0	3.9	6.3	4.4	4.6	0.9	1.4	2.5	3.5	3.3	5.3	2.2	1.8	0.9	1.4	*	1.0	1.4	1.8	4.4
*	0.6	6.4	*	1.0	8.2	0.1	1.4	0.2	1.3	0.4	2.8	0.6	2.5	0.3	0.7	0.2	1.5	0.4	7.2	0.3	0.3	0.2	0.4	0.2	0.3	0.8	0.2	2.0
0.2	2.5	14.6	0.1	0.5	2.3	0.4	2.1	1.3	5.1	3.8	7.2	4.0	5.7	0.8	1.6	2.5	5.0	0.3	5.7	0.8	2.1	0.6	1.8	0.7	0.6	1.5	1.4	5.4
0.3	3.5	16.1	0.3	0.6	2.2	0.6	2.0	1.6	5.8	4.7	7.7	3.5	5.9	1.0	1.9	2.9	5.5	0.5	6.2	1.0	2.2	0.8	1.9	0.7	0.5	1.4	1.9	6.1
0.5	1.4	6.4	0.1	0.4	3.7	0.2	1.2	0.7	2.3	2.5	4.4	1.6	4.2	0.8	1.0	1.6	2.3	0.2	3.4	0.6	0.3	0.5	0.7	0.3	0.8	1.2	0.7	3.1
0.5	1.7	5.9	0.1	0.4	4.3	0.2	0.9	0.6	2.1	2.1	4.1	1.4	3.9	0.8	0.9	1.4	2.1	0.3	3.2	0.6	0.2	0.4	0.6	0.3	0.8	1.2	0.7	2.8
0.1	15.1	15.9	0.0	1.4	1.4	2.0	1.9	3.1	4.0	4.6	5.0	6.0	5.9	1.4	1.4	3.4	3.6	7.5	8.0	4.3	2.0	2.0	1.7	*	0.4	0.4	4.7	5.6
0.4	5.1	8.4	0.0	0.8	1.1	0.5	1.1	1.2	2.7	2.7	4.6	4.0	4.6	0.9	1.2	1.4	1.9	2.9	5.7	1.8	0.2	0.9	0.5	*	1.5	1.6	1.6	3.4
*	0.9	16.0	*	0.2	1.7	0.4	1.9	1.1	5.9	4.7	7.9	4.5	5.9	1.1	2.2	2.7	5.5	0.3	7.1	0.8	2.1	0.5	1.9	0.8	0.5	1.6	1.3	6.3
0.3	0.2	7.9	0.05	0.5	16.8	0.3	1.0	0.5	1.6	0.9	3.2	2.0	3.3	0.3	0.8	1.1	2.4	0.1	9.7	0.2	1.8	0.1	0.9	*	0.5	0.6	0.3	2.3
*	*	0.2	*	*	0.1	*	*	*	0.1	*	0.1	*	0.1	*	*	*	0.1	0.1	0.1	*	0.1	*	*	*	*	*	*	0.1
0.4	0.6	7.4	0.01	0.2	3.4	0.1	1.2	0.3	2.3	1.6	4.6	0.8	4.0	0.5	1.0	1.3	2.7	0.1	4.7	0.3	0.8	0.2	0.9	0.3	0.8	2.2	0.3	2.9
*	0.7	8.1	*	0.7	15.9	0.2	0.8	0.6	2.1	1.6	3.8	2.2	3.4	0.3	0.7	1.4	2.8	0.3	8.8	0.4	1.5	0.3	1.1	0.3	0.5	0.6	0.7	2.8
0.4	0.3	5.9	0.01	0.2	1.5	0.3	0.8	0.2	1.3	0.8	2.3	1.2	2.4	0.2	0.2	0.2	1.4	0.1	1.8	0.2	0.5	0.1	0.5	*	0.2	0.8	0.1	1.5
0.5	0.4	6.5	0.04	0.2	1.8	0.1	0.9	0.2	1.6	0.9	2.7	1.5	2.8	0.2	0.3	0.3	1.6	0.1	1.9	0.3	0.6	0.1	0.6	0.1	0.3	1.0	0.1	1.7
0.3	0.9	12.6	0.06	0.5	3.0	0.4	2.1	1.1	3.8	3.9	6.2	3.6	6.2	1.0	1.9	2.4	3.6	0.3	5.4	0.7	2.1	0.7	1.9	0.6	0.7	1.6	1.3	4.7
0.4	0.7	8.4	0.02	0.2	8.2	0.1	1.3	0.3	3.3	1.4	5.7	0.8	4.2	0.3	1.4	1.0	3.6	0.1	4.6	0.2	1.7	0.2	1.5	0.1	0.6	1.8	0.2	3.7
0.4	0.4	6.3	0.05	0.4	7.3	0.1	0.8	0.4	2.0	1.4	4.2	0.9	3.4	0.6	1.0	1.1	2.3	0.1	6.3	0.2	0.3	0.1	0.7	0.1	0.5	1.2	0.4	2.8
1.0	1.8	7.5	0.08	0.9	6.2	0.3	1.3	1.1	3.7	3.3	6.2	2.5	4.2	0.8	1.2	2.2	3.9	0.5	3.8	0.8	1.9	0.6	1.7	0.5	0.7	1.3	1.0	4.0
0.6	1.2	8.0	0.04	0.4	6.5	0.1	1.3	0.6	3.2	2.3	5.6	1.5	4.2	0.6	1.3	1.5	3.5	0.3	3.8	0.5	1.6	0.4	1.5	0.3	0.8	1.6	0.5	3.5
0.3	0.6	6.5	0.02	0.2	5.9	0.1	1.1	0.3	2.2	1.2	4.3	0.7	4.0	0.5	1.1	0.9	2.3	0.1	5.0	0.2	0.4	0.2	0.8	0.2	0.5	1.6	0.2	2.9
0.5	0.7	6.9	*	0.1	2.2	0.5	1.3	0.9	2.6	2.2	3.9	2.6	3.4	0.4	0.3	1.3	2.4	0.2	2.6	0.3	1.2	0.5	1.0	0.1	1.0	0.8	1.0	2.8
0.4	0.4	8.9	0.1	0.2	2.1	0.2	1.1	0.6	2.8	1.7	4.3	1.9	2.9	0.3	0.5	1.2	3.1	0.2	2.0	0.3	1.4	0.2	1.1	0.2	1.3	1.3	0.4	2.7
0.5	2.2	4.0	0.5	0.2	1.6	1.0	2.0	2.6	5.7	6.4	7.2	6.0	7.4	1.6	1.7	3.1	3.7	0.7	6.7	1.1	1.7	1.3	2.5	1.2	0.6	0.9	3.1	6.0
0.2	7.3	10.6	0.2	1.5	3.0	0.6	1.5	2.1	3.2	3.0	4.0	2.5	5.1	0.8	0.9	2.4	2.9	1.1	2.0	1.7	1.5	1.5	1.7	0.9	0.1	0.9	2.7	4.0
0.2	6.6	8.7	0.3	1.3	2.7	0.5	1.1	2.1	3.6	3.5	4.9	2.3	4.2	0.8	0.8	2.3	3.3	0.9	1.8	1.5	1.4	1.3	1.4	0.6	0.8	0.9	2.4	3.7
0.3	1.1	13.2	0.1	0.1	6.3	0.5	4.8	1.1	8.3	5.3	10.4	3.3	10.6	1.1	2.5	2.7	7.1	0.2	10.9	0.4	2.5	0.6	2.4	0.8	0.4	1.6	1.5	9.1
0.3	1.4	15.1	0.05	0.2	1.7	0.5	1.9	1.3	5.5	4.8	7.5	5.5	6.2	1.0	2.1	3.0	5.2	0.2	6.6	0.7	2.2	0.7	1.8	0.8	0.5	1.3	1.7	5.9
0.4	0.7	9.8	0.02	0.1	1.4	0.6	1.6	1.1	3.8	4.2	6.0	5.4	5.9	0.7	1.4	2.8	3.4	0.1	7.3	0.7	0.3	0.7	0.8	0.8	0.4	1.2	1.5	4.6
0.4	1.3	10.6	*	0.4	4.4	0.3	1.5	1.0	4.0	3.5	6.4	3.5	4.9	0.9	1.6	2.2	4.0	0.4	4.8	0.7	1.8	0.6	1.6	0.5	0.6	1.4	1.3	4.4
0.2	7.3	11.1	0.1	1.6	3.3	0.3	1.4	2.5	4.7	4.0	6.2	2.7	4.9	0.9	1.1	2.7	4.4	1.3	2.3	1.3	1.9	1.7	1.8	0.7	0.9	1.2	3.0	4.8
0.2	6.6	9.4	0.2	1.0	3.0	0.4	1.3	1.8	3.0	3.0	4.1	1.9	4.6	0.6	0.8	2.0	2.6	0.8	2.0	1.3	1.6	1.1	1.6	0.5	0.8	1.2	2.2	3.5
0.2	6.4	9.6	0.35	1.3	2.8	0.5	1.2	2.1	2.8	3.5	3.9	2.4	4.2	0.8	0.8	2.3	2.5	0.9	1.9	1.5	1.5	1.4	1.5	0.6	0.7	0.8	2.5	3.3
0.2	6.8	10.4	0.3	1.3	2.9	0.6	1.2	1.8	3.8	2.8	4.7	2.2	4.6	0.7	0.8	2.1	3.6	0.9	1.9	1.6	1.7	1.3	1.6	0.5	0.5	0.8	2.4	4.1

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