



Acumedia Manual

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Acumedia Dehydrated Culture Media

Listed in alphabetical order

A-1 Medium	Dermatophyte Test Medium
Agar, Bacteriological	Dextrose Tryptone Agar
APT Agar	Dextrose Tryptone Broth
Azide Dextrose Broth	Dichloran Glycerol (DG-18) Agar Base
Bacillus Cereus Agar Base	Dipeptone
Baird Parker Agar	DNase Test Agar
BCYE Agar (Legionella Agar)	DNase Test Agar w/ Methyl Green
Beef Extract Powder	DRBC Agar
Beta-SSA Agar	EC Medium
BIGGY Agar	EC Medium w/ MUG
Bile Esculin Agar	EC Medium, Modified
Bile Esculin Azide Agar	Elliker Broth
Bile Salts Mixture # 3	EMB Agar (Holt, Harris & Teague)
Bismuth Sulfite Agar	EMB Agar, Levine
Blood Agar Base No.2	Eugonic Agar
Blood Agar Base w/ Low pH	Fastidious Anaerobe Agar
Blood Agar Base, Improved	Fluid Thioglycollate Medium
Blood Agar Base, pH 7.4	Fraser Broth
Brain-Heart Infusion Agar	Fraser Broth Base
Brain-Heart Infusion Broth	Fungisel Agar
Brain-Heart Infusion Solids	GC Agar
Brain-Heart Infusion w/o Dextrose	Gelatin
Brilliant Green Agar	GN Broth (Hajna)
Brilliant Green Agar w/ Sulfadiazine	HC Agar Base
Brilliant Green Agar w/ Sulfapyridine	Heart Infusion Agar
Brilliant Green Bile Agar	Heart Infusion Broth
Brilliant Green Bile Broth 2%	Hektoen Enteric Agar
Brilliant Green Bile Broth 2% w/ MUG	Hemoglobin Powder
Brucella Agar	Inhibitory Mold Agar
Brucella Broth	Kligler Iron Agar
Buffered Listeria Enrichment Broth	Lactobacilli MRS Agar
Buffered Peptone Water	Lactobacilli MRS Broth
Campy Blood Free Selective Medium (CCDA)	Lactobacillus Selective Agar Base
Campy Selective Agar Base (Preston)	Lactose Broth
Campylobacter Enrichment Broth	Lauryl Sulfate Broth
Cary and Blair Transport Medium	Lauryl Sulfate Broth w/ MUG
Cary and Blair Trans Med, Mod w/ Phenol Red	LB Agar (Lennox L Agar)
Casein, Acid Hydrolysate	LB Broth (Lennox L Broth)
Casman Medium Base	Lethen Agar Base
CDC Anaerobe Agar	Lethen Agar Base, Modified
Cetrimide Agar	Lethen Broth Base
Charcoal Agar	Lethen Broth Base, Modified
CHO Medium Base	Listeria Enrichment Broth
CLED Agar	Littman Agar
Clostridium Difficile Agar	Lowenstein-Jensen Medium
Columbia Blood Agar Base	LPM Agar
Columbia Broth	Luria Agar (Miller's LB Agar)
Columbia CNA Agar	Luria Broth Base (Miller's LB Broth)
Cooked Meat Medium	Lysine Iron Agar
D/E Neutralizing Agar	M-Broth
D/E Neutralizing Broth	m-Enterococcus Agar
Deoxycholate Agar	m-FC Agar
Deoxycholate Citrate Agar	m-FC Broth

m-Green Yeast and Fungi Broth	Sabouraud BHI Agar
m-TEC Agar	Sabouraud Dextrose Agar
m-TGE Broth	Sabouraud Dextrose Agar w/ Chloramphenicol
M17 Broth Base	Sabouraud Dextrose Agar w/ Lec & Tween 80
MacConkey Agar	Sabouraud Dextrose Agar, Emmons
MacConkey Agar w/o Crystal Violet	Salmonella Shigella Agar
MacConkey Agar w/o Crystal Violet & Salt	Schaedler Agar
MacConkey Agar w/ Sorbitol	Schaedler Broth
MacConkey Agar, CS	Selective Strep Agar, Modified #2
MacConkey Agar, Modified	Selenite Broth
MacConkey Broth	Selenite Cystine Broth
Malonate Broth	SIM Medium
Malt Agar	Simmons Citrate Agar
Malt Extract	Skim Milk
Malt Extract Agar	Soy Peptone Yeast Extract Agar
Mannitol Salt Agar	Standard Methods Agar
MCP Agar	Staph Selective Agar
Middlebrook 7H10 Agar	Staphylococcus Agar # 110
Middlebrook 7H11 Agar	Sterility Test Broth
MIO Medium	TAT Broth
Mitis Salivarius Agar	TCBS Agar
Motility Test Agar	Tergitol 7 Agar
MRSA Agar Base	Tetrathionate Broth Base
MR-VP Broth	Thioglycollate Medium w/o Indicator
Mueller Hinton Agar	Todd Hewitt Broth
Mycobiotic Agar	Tomato Juice Agar
Mycological Agar	Triple Sugar Iron Agar
Nutrient Agar	Tryptic Soy Agar
Nutrient Agar 1.5%	Tryptic Soy Agar w/ Lec & Tween 80
Nutrient Broth	Tryptic Soy Broth
Nutrient Gelatin	Tryptone
Orange Serum Agar	Tryptone Glucose Extract Agar
Ox bile (Ox gall)	Tryptose Agar
Oxford Listeria Agar Base	Tryptose Blood Agar Base
Pancreatic Digest of Casein (Peptone C)	Tryptose Broth
Pancreatic Digest of Gelatin (Peptone G)	Tryptose Phosphate Broth
Papaic Digest of Soybean Meal (Peptone S)	Universal Beer Agar
Peptic Digest of Animal Tissue (Peptone A)	Universal Pre-Enrichment Broth
Peptone Water	Urea Agar Base
Phenol Red Broth Base	UVM Mod Listeria Enrichment Broth
Phenylethanol Agar	Violet Red Bile Agar
Phosphate Buffer, pH 7.2	Violet Red Bile Agar w/ MUG
Potato Dextrose Agar	Violet Red Bile Glucose Agar
Potato Dextrose Agar with Lec & Tween 80	Vogel and Johnson Agar
Potato Dextrose Broth	Wilkins-Chalgren Agar
Potato Infusion Agar	Wilkins-Chalgren Broth
Presence-Absence Broth	W-L Nutrient Medium
Pseudomonas F Agar	XL Agar Base
Pseudomonas Isolation Agar	XLD Agar
Pseudomonas Isolation Broth	XLT4 Agar
Pseudomonas P Agar	Yeast Enriched Peptone
Purple Lactose Agar	Yeast Extract
R2A Agar	Yersinia Selective Agar
Rappaport-Vassiliadis (MSRV) Medium	YM Agar
Semisolid Modified	YM Broth
Rappaport-Vassiliadis R10 Broth	

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Introduction

One of the basic characteristics of a microorganism is its nutritional requirements. A **dehydrated culture medium** is described as a substance or a group of substances that satisfies those nutritional requirements. With many simple and complex formulations of dehydrated culture media available, it is important for a media manufacturer to produce the finest product possible.

Since 1978, Acumedia has been recognized as a premier manufacturer of high-quality dehydrated culture media for industrial, biotech and life science applications. In 2000, Acumedia became a wholly-owned subsidiary of Neogen Corporation, a world leader in rapid diagnostic systems for bacteria, natural toxins, food allergens, drugs and other important diagnostic areas. As a part of Neogen, Acumedia is now able to heighten its commitment to quality products, technical support, and personalized customer service.

Although Acumedia is now a subsidiary of Neogen, dehydrated culture media remains all that we do. Through our state-of-the-art manufacturing facility in Baltimore, we are able to offer high-value dehydrated culture media of superior and consistent quality, as well as unparalleled service to our customers. We possess the manufacturing capacity to produce standard or custom blends to fill the needs of high-volume large-scale industrial applications. Yet, we are able to address all aspects of providing our customers with product of high value, no matter how it is defined—be it quality, delivery, availability, service or cost.

Quality

Using only the finest ingredients from approved vendors, Acumedia's raw ingredients must pass stringent QC analyses before they are milled and blended to produce superior homogeneous dehydrated culture media. Detailed certificates of origin are available for every product and lot number. Acumedia's look has been updated to reflect a change in our business philosophy. We've held on to what our customers have trusted, and added the flexibility to change for the better. Our new "wide mouth" bottles and enhanced appearance are small indications of all we are doing to become the standard of quality in dehydrated culture media manufacturing.

Service

Acumedia is fully committed to maintaining the inventory necessary to fill a customer's needs—on the customer's timetable. Having our varied media products in stock, and continuously available for same day and next-day shipments, is what our customers have come to expect from us, and what we expect from ourselves. Should questions arise about the correct usage of any of our products, we stand behind them with around-the-clock professional technical support.

Value

Acumedia is committed to being the industry leader in service, while maintaining its reputation as a producer of high-quality products. Acumedia provides the greatest value dehydrated culture media by supplying:

- Consistency from lot-to-lot
- Availability
- On-time delivery
- Outstanding customer and technical service

Our goal is to provide quality, service and value unmatched in the dehydrated culture media industry.

Growth Requirements for Microorganisms

To support microorganism growth in the laboratory, it is necessary to establish conditions that will permit organism reproduction. All microorganisms require the following to remain viable and grow on culture media:

Complex Nutrients

Microorganisms utilize nitrogen and carbon through the addition of peptones, beef extract, and yeast extract to culture media. Specific nutritional requirements for different microorganisms vary greatly, but every microorganism requires sources of carbon, nitrogen, inorganic phosphate, sulfur, trace metals, water, and vitamins. All of these requirements also comprise a satisfactory microbiological culture medium. Buffering agents, indicators of pH change, selective agents, and agar are also added.

Proper pH

A large number of culture media are prepared with a final neutral pH of 7.2 ± 2 . The microorganisms that prefer a neutral pH are referred to as neutrophiles, or neutral-loving microorganisms. The bulk of human pathogens are within this group. Acidophiles, or acid-loving microorganisms, prefer a pH of 0.0 – 5.4. Yeast and molds are acidophiles. Alkaliphiles, alkali-loving microorganisms, are viable in a pH of 7.0 – 11.5. *Vibrio cholerae* is an alkaliphile.

Appropriate Temperature

Mesophilic bacteria and fungi have optimal growth at temperatures of 25 - 40°C. The vast majority of human pathogens are mesophilic, because they prefer body temperature. Thermophilic microorganisms (heat-loving) grow at temperatures greater than 45°C. *Bacillus stearothermophilus* is an example of a thermophilic microorganism, and can be found in hot spring beds. Psychrophilic microorganisms grow at temperatures below 20°C. *Listeria spp.* are psychrophilic microorganisms, and can be isolated from ice cream and other dairy products.

Gases

Obligate aerobes require the presence of oxygen to grow. Anaerobes grow only in the absence of oxygen. Microaerophiles prefer partial anaerobic conditions, and facultative anaerobes are capable of growing in the presence or absence of oxygen. Many microorganisms require an environment of 5 - 10% CO₂.

Moisture

Proper moisture conditions are important for proper microorganism growth. Water must be able to flow freely in and out of cells for transfer of nutrients and waste products. Evaporation during incubation or storage results in loss of water and microorganism reduction.

Media Components

Culture media are formulated to create appropriate environments for specific microorganisms. Some common media constituents include:

Amino-Nitrogen

Peptones, hydrolysates, infusions, and extracts are the main sources of nitrogen, essential for microorganism reproduction and metabolism.

Growth Factors

Sheep, horse and rabbit blood, serum, and vitamins are added to support or enhance microorganism growth.

Source of Energy

Carbohydrates and alcohols are added as carbon and energy sources which stimulate growth of microorganisms. Carbohydrates are also used to aid in microorganism identification and differentiation.

Buffering Agents

Phosphates, acetates, and citrates maintain the pH in culture media.

Minerals

Phosphate, sulfate, magnesium, calcium, manganese, and iron salts provide trace elements.

Selective Agents

Antimicrobials, dyes, and bile salts are used to restrict the growth of certain organisms, while permitting the growth of others.

Indicator Dyes

Dyes, such as phenol red and bromthymol blue, are used in the preparation of differential and selective culture media.

Gelling Agents

Agar and gelatin are added to a liquid medium to change the consistency to a solid or semisolid medium.

Media Ingredients, Peptones and Hydrolysates

Beef Extract Powder

Beef Extract Powder is a replacement for infusion of meat, and is standard in composition and reaction. Beef Extract Powder provides sources of nitrogen, vitamins, amino acids and carbon.

Bile Salts Mixture #3

Bile Salts Mixture #3 is used as a selective agent for the isolation of Gram-negative microorganisms, inhibiting Gram-positive cocci. Bile is derived from the liver. Bile Salts Mixture #3 contains bile extract standardized to provide inhibitory properties for selective media.

Brain-Heart Infusion Solids

Brain-Heart Infusion (BHI) Solids is composed of a dehydrated infusion of porcine brains and hearts for use in the preparation of culture media. BHI Solids provides nitrogen, amino acids, and vitamins. BHI Solids is processed from large volumes of raw material, retaining all the nutritive and growth stimulating properties of fresh tissues.

Casein, Acid Hydrolysate

Casein is a milk protein and a rich source of amino nitrogen. Casein, Acid Hydrolysate, a hydrochloric acid hydrolysate of casein, is added to media primarily because of the organic nitrogen and growth factor components. Casein, Acid Hydrolysate is recommended for use in microbiological cultures that require completely hydrolyzed protein as a nitrogen source.

Dipeptone

Dipeptone is a blend of enzymatic digest of animal tissue and casein. Dipeptone contains many peptide sizes in combination with vitamins, nucleotides, minerals and other carbon sources. Dipeptone is particularly well suited in supplying the growth requirements of fastidious bacteria. This peptone is extremely valuable in media for cultivation of pathogenic fungi. Growth of these microorganisms is rapid and colony formation is uniform and typical.

Gelatin

Gelatin is a protein of uniform molecular constitution, and derived chiefly from the hydrolysis of collagen. Collagens are a class of albuminoids, found abundantly in bones, skin, tendon, cartilage and similar tissues of animals. Gelatin is used in culture media to determine protease production by bacteria, and as a nitrogen and amino acid source.

Hemoglobin Powder

Hemoglobin Powder is freeze-dried hemoglobin for use in preparing microbiological culture media. Hemoglobin Powder is used with GC Agar to provide an enriched medium for the isolation and cultivation of fastidious microorganisms. Hemoglobin Powder provides the hemin (X factor) required for growth of *Haemophilus*, and for enhanced growth of *Neisseria* spp.

Malt Extract

Malt Extract is obtained from barley, and designed for the propagation of yeasts and molds. Malt Extract is well suited for yeasts and molds due to a high concentration of carbohydrates, particularly maltose. This product is generally employed in concentrations of 1 - 10%, and provides carbon, protein and other nutrients.

Oxbile (Oxgall)

Oxbile is manufactured from large quantities of fresh bile by rapid evaporation of the water content. Bile is composed of fatty acids, bile acids, inorganic salts, sulfates, bile pigments, cholesterol, mucin, lecithin, glycuronic acids, porphyrins and urea. The use of Oxbile insures a consistent supply of bile, and assures a degree of uniformity impossible to obtain with fresh materials. It is prepared for use in selective media for differentiating groups of bile-tolerant bacteria. Oxbile is used as a selective agent for the isolation of Gram-negative microorganisms, inhibiting Gram-positive bacteria. The major composition of Oxbile is taurocholic and glycocholic acids.

Pancreatic Digest of Casein (Peptone C)

Pancreatic Digest of Casein is recommended for preparing media where an enzymatically hydrolyzed casein is desired. Casein is a rich source of amino nitrogen. This product is used to support the growth of fastidious microorganisms, and has a high tryptophan content.

Pancreatic Digest of Gelatin (Peptone G)

Pancreatic Digest of Gelatin is deficient in carbohydrates. Pancreatic Digest of Gelatin is used as a media ingredient for fermentation studies, and alone to support the growth of non-fastidious microorganisms.

Papaic Digest of Soybean Meal (Peptone S)

Papaic Digest of Soybean Meal is a nitrogen source, and contains the naturally occurring high concentrations of vitamins and carbohydrates of soybean. Papaic Digest of Soybean Meal minimizes bovine spongiform encephalopathy (BSE) risk in vaccine production because the origin of this product is plant.

Peptic Digest of Animal Tissue (Peptone A)

Peptic Digest of Animal Tissue provides sources of nitrogen, amino acids, vitamins and carbon in microbiological culture.

Skim Milk

Skim Milk is a soluble, spray-dried skim milk. This product is used as a complete medium or incorporated into other media for the isolation and cultivation of microorganisms found in milk products. Skim Milk is also used for differentiating organisms based on coagulation and proteolysis of casein.

Tryptone

Tryptone is an enzymatic digest of casein used as a nitrogen source in culture media. Casein is the main protein of milk, and a rich source of amino-acid nitrogen. Tryptone is rich in tryptophan, making it valuable for use in detecting indole production. The absence of detectable levels of carbohydrates in Tryptone makes it a suitable peptone in differentiating bacteria on the basis of their ability to ferment various carbohydrates.

Yeast Enriched Peptone

Yeast Enriched Peptone is an enzymatic digest of casein and yeast extract and is rich in vitamins and carbohydrates.

Yeast Extract

Yeast Extract is the water soluble portion of autolyzed yeast. Yeast Extract is an excellent stimulator of bacterial growth. The autolysis is carefully controlled to preserve the naturally occurring B-complex vitamins. Yeast Extract is generally employed in concentrations of 0.3 - 0.5%. Yeast Extract also provides vitamins, nitrogen, amino acids and carbon.

Culture Media Descriptions

General Purpose Media

General purpose media are designed to grow most organisms and do not contain growth inhibitors. Standard Methods Agar and Blood Agar Bases are examples of general purpose media.

Differential Media

Differential media contain a component that allows an observable change when a specific chemical reaction takes place. Simmons Citrate Agar is an example of a differential medium. In Simmons Citrate Agar there is a pH indicator that turns from green to blue when citrate is utilized as the sole carbon source.

Selective Media

Selective media encourage the growth of some organisms and suppress the growth of others. Dyes, antimicrobials, and salts are all examples of selective agents used for this purpose. Bile Salts are used to inhibit the growth of Gram-positive organisms on MacConkey Agar.

Selective / Differential Media

A medium can be both selective and differential depending on the formula. Hektoen Enteric Agar is an example of a selective and differential medium. Hektoen Enteric Agar contains Bile Salts, the selective agent used to suppress Gram-positive organisms, and pH indicators, Acid Fuchsin, and Bromthymol Blue.

Enrichment Media

Enrichment media contain nutrients that encourage the growth of organisms. Media containing enrichments can be selective or general purpose. Universal Pre-enrichment Broth is a general purpose enrichment broth for *Salmonella* and *Listeria*, and is particularly successful at resuscitating low numbers of injured cells.

Chemically Defined Media

Chemically defined media are made of specific types and amounts of pure chemicals. Tissue culture media are examples of chemically defined basal formulations.

Agar

Agar is the technical name of the gelling agent most commonly used in culture media. The term “agar” frequently refers to a solid surface, plated medium. Plated media are used for isolating pure colonies or counting numbers of colonies. Organism identification is possible after obtaining pure colonies.

Agar Slants

Agar containing media that have solidified in tubes in a slanted position are referred to as slants. Triple Sugar Iron Agar is an example of a medium that is prepared in slants and used for organism identification.

Broths

Broths are referred to as any liquid culture medium. Tryptic Soy Broth is an example of a widely used broth formula.

Semisolid Media

Semisolid media are between a solid and a liquid state. Agar added in low concentrations achieve fluidity but not at a concentration that will result in a firm gel. Motility Test Medium is an example of a semisolid medium. Bacterial motility may be observed macroscopically as a diffuse zone of growth spreading from the line of inoculation.

Use of Dehydrated Culture Media

Receipt of Dehydrated Culture Media

Upon arrival in the laboratory, dehydrated culture media should be labeled with the date of receipt. Dehydrated culture media are hygroscopic and sensitive to heat, light, and moisture.

1. Store media as directed on label, usually below 30°C in a dry area, away from direct sunlight, autoclaves, drying ovens, or other heat sources. When indicated, store at 2 - 8°C.
2. Check expiration date on the label. Expiration dates vary depending upon the culture media.
3. If possible, use inventory in lot/batch number order. When the container is opened for the first time, note this date on the label. After use, make sure the container is tightly closed and returned to the proper storage area.
4. Discard medium if the powder is not free flowing, or if its appearance has changed from its original color or consistency.

Reconstitution of Dehydrated Culture Media

Directions for the preparation of culture media are provided on the label of each bottle. Follow these directions carefully, using the guidelines below.

1. Dehydrated culture media should be prepared with purified water, having a neutral pH.
2. Rinse all glassware before use to remove any trace substances, especially detergents. Prepare the medium in a flask that holds twice the final volume of the medium to allow adequate mixing.
3. Weigh out the appropriate amount of dehydrated medium quickly and accurately. Avoid creating dust. Do not inhale powder. Close the container as soon as possible to prevent contamination.
4. Add half of the required volume of water in the flask, followed by the weighed quantity of medium. Agitate briskly for a few minutes until a homogeneous suspension is obtained. Add the remaining water, ensuring any medium that has adhered to the wall of the flask is added to the liquid.
5. Culture media without agar or gelatin (broth formulations) can usually be dissolved with gentle heat and agitation. Culture media containing agar or gelatin must be heated with frequent agitation and boiled for one minute to completely dissolve the medium. **Caution:** Overheating culture media containing agar will cause it to boil over very rapidly.
6. After adequate cooling, media that should not be autoclaved are ready to pour into petri dishes or tubes. Most culture media require sterilization in an autoclave. Refer to label directions.

Sterilization

The recommended steam sterilization cycle is 15 minutes at 121°C for 1 liter or less. Larger volumes may require longer cycles. Autoclaves vary in performance and thermocouple tests using volumes of media should be carried out to verify heating and cooling times.

pH Adjustments

Commercial dehydrated culture media are prepared so the final pH conforms with the label specification. The pH should be tested after the medium has cooled to 25°C and solidified. For filter sterilization, adjust the pH if necessary, prior to filtering. Avoid excessive pH adjustments because this can alter the chemical composition of the medium.

Supplements

Add supplements to media cooled to 45 - 55°C, because they are frequently heat sensitive. Swirl after addition to ensure adequate mixing. Sterile broths may be cooled to room temperature before adding supplements. Sterile blood used for the preparation of blood agar should be fresh and stored at 2 - 8°C. Warm blood in an incubator (35 - 37°C) before adding to sterile medium. Defibrinated blood is recommended for use rather than blood containing an anticoagulant.

Dispensing Media

1. Medium should be cooled in a water bath to 50 - 55°C prior to dispensing.
2. Gently swirl medium before and during dispensing to ensure that it is evenly mixed. Dispense quickly.
3. Immediately recap or recover tubes to reduce the chance of contamination. Petri dish covers should be slightly ajar for 1 – 2 hours to reduce moisture build-up on lids.

Storage

The recommended expiration date of prepared culture media varies greatly. Screw-capped tubes can be stored for 6 months or longer at low to ambient temperatures. Plated media may be stored inverted in a plastic bag or other container in a refrigerator for 1 – 2 weeks or longer. Store all media away from light.

It is good laboratory practice to establish expiration dates for all prepared media formulations, record the information for future lot numbers and documentation, and date the containers. Internal environments vary greatly and can affect media performance and appearance. Dispose of prepared media if contamination occurs, appearance changes, hemolysis is present, or signs of dehydration including shrinking or cracking appear.

Quality Control

Consistent, documented quality control procedures and results are essential for every laboratory. Quality control tests ensure culture media are prepared according to label directions, and performance characteristics are within specification. Each lot / batch number of medium that is prepared in-house should be tested for the following parameters:

1. pH value: Check pH of prepared medium (after cooled to 25°C) to ensure the pH falls within the range on the product label.
2. Sterility: A representative sample of each lot / batch number of medium should be incubated for 2 – 5 days at 35°C. There should be no evidence of microbial growth after incubation.
3. Growth performance: Test each lot / batch number of medium to ensure it will support the growth of appropriate microorganisms. Inoculate medium with appropriate stock cultures, and if required, perform necessary biochemical tests. Comparing old / new lot numbers is extremely helpful.
4. Stability: Periodically perform the above procedures on stored prepared media to determine that storage conditions will give optimal results.

Certificates of Analysis are obtainable for every formula and lot number of dehydrated culture media available from Acumedia. Certificates of Analysis contain the minimum quality control specifications for each lot number of dehydrated culture media.

If a prepared medium does not perform as expected please inform Technical Service. Record the nature of the problem and method of preparation. Note the lot / batch number and the date prepared and received.

Precautions

Dehydrated culture media from Acumedia are For In Vitro Diagnostic Use, For Laboratory Use or For Manufacturing Use as labeled. Follow usual precautionary measure for handling chemicals when working with dehydrated culture media. Keep all culture media away from food and beverages. Immediately remove soiled and contaminated clothing. Avoid contact with skin and eyes.

Observe aseptic techniques and follow all laboratory precautions against microbiological hazards when performing any procedure. All samples should be treated as infectious. The use of a biohazard cabinet is recommended when working with pure cultures or samples suspected of containing fungi, brucellae, or mycobacteria. Follow universal precautions when handling blood or blood products.

Disposal

After use, prepared plates, samples, sample containers, or other contaminated materials must be sterilized or incinerated before discarding. Directions for use of the incinerator or autoclave must be followed carefully. Dispose of all autoclaved biohazards in accordance with applicable federal, state, and local environmental regulations.

Media Inoculation

Streak Method for Agar Plates

The streak plate is used primarily for isolating microorganisms in pure culture from specimens or samples containing mixed flora. Obtaining isolated colonies on plates allows colonial morphology and hemolytic reactions to be examined, and biochemical / serological testing/ to be performed.

1. With a sterile inoculating loop, streak a loopful of the sample across the surface of an agar plate. The four-quadrant streak is the most common, and accomplished by streaking and rotating the plate in four sections, one quarter at a time, slightly overlapping the original streak area. The fourth quadrant contains the greatest dilution of microorganisms, and usually provides isolated colonies for further testing.
2. Incubate plates under favorable growth conditions.
3. Examine plates for isolated colonies.

Spread Plate Technique

The spread plate technique is used for enumerating microorganisms.

1. Drop 0.1 mL aliquots from serial dilutions onto the surface of an agar plate.
2. Aseptically spread inoculum across the surface using a bent glass rod or sterile inoculating loop. By spreading the suspension over the plate, a dilution gradient is established to provide isolated colonies.
3. Incubate plates agar inverted in appropriate conditions.
4. Count colonies and calculate the number of microorganisms in the original suspension.

Pour Plate Technique

The pour plate technique is also used for enumeration of microorganisms in a particular sample. In this technique, test samples or suspensions of microorganisms are mixed with molten agar (45-50°C). The agar is allowed to solidify, trapping the bacteria at separate discrete positions within the matrix of the medium. While the medium holds bacteria in place, it is soft enough to permit growth of bacteria and the formation of discrete isolated colonies.

1. Perform serial dilution of sample.
2. Aseptically pipette microorganism dilutions into labeled petri dishes.
3. Add melted agar that has been cooled to approximately 44 - 45°C.
4. Mix well by slightly rotating plate with bacteria and agar mixture.
5. Allow the agar to solidify, trapping bacteria at separate discrete positions within the medium.
6. Incubate plates in a favorable environment.
7. Count the number of colonies and calculate the number of microorganisms in the original sample.

Streak / Stab Method for Agar Tubes

Tubed media may be in the form of solid agar slants, semisolids, or broths. Depending on the type of medium used and the purpose of the inoculation, use an inoculating loop or needle.

1. For agar slants, place the loop at the base of the tube surface and draw it up the agar surface while moving it from side to side.
2. For semisolid media, insert the loop into the medium to approximately one-fourth of its depth. If testing motility, use an inoculating needle and stab it in the center of the agar tube to the bottom. Draw the needle out carefully, keeping it straight.

Inoculation of Broth Media

Broth media are generally used as enrichments, general cultivation and sterility testing.

1. Aseptically inoculate appropriate broth media with the sample or specimen using sterile pipette, syringes or forceps.
2. Incubate inoculated broth at the appropriate atmospheric conditions, temperature, and time.
3. Examine broth for any signs of growth including, turbidity with or without gas bubbles, "puff-ball" appearance, hemolysis (in blood cultures), pellicle formation and precipitate on the bottom of the tube or bottle.

Membrane Filtration Method

The membrane filtration method is used to test large volume of liquid samples, including water and filterable beverages.

1. Pass the sample through a sterile membrane filter enclosed in a filtration assembly and attached to a vacuum source.
2. After filtering the sample, carefully remove the filter with sterile forceps and apply it to the surface of an agar plate or pad saturated with a broth medium. Avoid trapping air bubbles by using a rolling action. (The media used depends on the type of microorganism being tested.)
3. Invert plates and incubate under appropriate conditions.
4. Count colonies and calculate the most probable number.

Troubleshooting Guide

Troubleshooting errors in the preparation of dehydrated culture media

	<i>Deteriorated medium</i>	<i>Improperly washed glassware</i>	<i>Impure water</i>	<i>Overheating</i>	<i>Repeated remelting</i>	<i>Incorrect dilution</i>	<i>Incomplete mixing/incorrect weighing</i>
Incorrect pH range	X	X	X	X	X		X
Nontypical precipitate	X	X	X	X			X
Abnormal medium color	X	X	X	X			X
Incomplete solubility							X
Carmelization or darkening	X			X	X		X
Toxicity		X	X				
Trace substances		X	X				
Medium will not solidify				X		X	X
Loss of nutrients/properties	X		X	X	X	X	X

Improper sterilization, poor technique in adding enrichments and pouring plates, and improper boiling of medium are also sources of contamination.

Product Category References

Fermentation Products	Product #	Anaerobes	Product #
Beef Extract Powder	7228	Brain-Heart Infusion Agar	7115
Brain-Heart Infusion Solids (Porcine)	7262	CDC Anaerobe Agar	7486
Brucella Broth	7121	CHO Medium Base	7563
Casein, Acid Hydrolysate	7229	Clostridium Difficile Agar	7385
Dipeptone	7183	Cooked Meat Medium	7110
Heart Infusion Broth	7319	Fastidious Anaerobe Agar	7531
M-Broth	7296	Fluid Thioglycollate Medium	7137
Malt Extract	7341	Schaedler Agar	7153
Pancreatic Digest of Casein (Peptone C)	7179	Schaedler Broth	7154
Pancreatic Digest of Gelatin (Peptone G)	7182	Thioglycollate Medium w/o Indicator	7160
Papaic Digest of Soybean Meal (Peptone S)	7180	Wilkins-Chalgren Agar	7232
Todd Hewitt Broth	7161	Wilkins-Chalgren Broth	7233
Tryptone	7351		
Tryptic Soy Broth	7164	Yeast and Mold	Product #
Tryptose Phosphate Broth	7278	Dichloran Glycerol (DG-18) Agar Base	7592
Universal Beer Agar	7574	DRBC Agar	7591
W-L Nutrient Medium	7488	Fungisel Agar	7205
Yeast Extract	7184	Malt Agar	7456
		Malt Extract Agar	7303
Molecular Genetics	Product #	m-Green Yeast & Fungi Broth	7190
LB Agar (Lennox L Agar)	7289	Mycological Agar	7309
LB Broth (Lennox L Broth)	7290	Potato Dextrose Agar	7149
Luria Agar (Miller's LB Agar)	7213	Potato Dextrose Broth	7585
Luria Broth Base (Miller's LB Broth)	7279	Sabouraud Dextrose Agar	7150
		YM Agar	7525
Sterility	Product #	YM Broth	7363
Fluid Thioglycollate Medium	7137		
Thioglycollate Medium w/o Indicator	7160		
Tryptic Soy Broth	7164		
Pharmaceutical	Product #		
APT Agar	7302		
Fluid Thioglycollate Medium	7137		
Tryptic Soy Broth	7164		
Environmental Sampling and Disinfectant Testing	Product #		
D/E Neutralizing Agar	7375		
D/E Neutralizing Broth	7562		
Lethen Agar Base	7118		
Lethen Broth Base	7105		
Pseudomonas Isolation Agar	7329		
Sabouraud Dextrose Agar	7150		
Sabouraud Dex Agar w/Lec & Tween 80	7392		
Standard Methods Agar	7157		
Tryptic Soy Agar	7100		
Tryptic Soy Agar w/Lec & Tween 80	7163		

Market-Specific Information

Clinical

	Anaerobes	Brucella	Gen. Purpose Culture Media	Gram (-) Enteric Bacteria	Pseudomonas	Salmonella/Shigella	Staphylococcus	Streptococcus	Yeast and Mold	Mycobacteria	Campylobacter
Baird Parker Agar (7112)						X					
Beta-SSA Agar (7336)							X				
BIGGY Agar (7191)								X			
Bile Esculin Agar (7249)							X				
Bile Esculin Azide Agar (7133)							X				
Bismuth Sulfite Agar (7113)					X						
Blood Agar Base No. 2 (7266)			X								
Brain Heart Infusion Agar (7115)	X		X					X			
Brilliant Green Agar (7449)					X						
Brucella Agar (7120)		X									
Campy Selective Agar (Preston) (7443)										X	
Campy Blood Free (CCDA) (7527)										X	
CDC Anaerobe Agar (7486)	X										
Cetrimide Agar (7222)				X							
Clostridium Difficile Agar (7385)	X										
Cooked Meat Medium (7110)	X										
Columbia Blood Agar Base (7125)			X								
Deoxycholate Agar (7130)				X							
Dermatophyte Test Medium (7265)								X			
Eosin Methylene Blue Agar (7134)				X							
EMB, Levine (7103)				X							
Eugonic Agar (7135)	X										
Fastidious Anaerobe Agar (7531)	X										
Fluid Thioglycollate Medium (7137)	X		X								
Fungisel Agar (7205)								X			
Heart Infusion Agar (7269)			X								
Hektoen Enteric Agar (7138)					X						
Inhibitory Mold Agar (7238)								X			
Lowenstein-Jensen Medium (7245)									X		
MacConkey Agar (7102)				X							
MacConkey Agar, CS (7391)				X							
Malt Agar (7456)								X			
Malt Extract Agar (7303)								X			
Mannitol Salt Agar (7143)						X					
Middlebrook 7H10 Agar (7246)									X		
Middlebrook 7H11 Agar (7244)									X		
MRSA Agar Base (7420)						X					
Mycobiotic Agar (7419)								X			
Mycological Agar (7309)								X			
Nutrient Agar (7145)			X								
Phenylethanol Agar (7147)						X	X				
Potato Dextrose Agar (7149)								X			
Potato Infusion Agar (7532)		X									

Clinical (cont.)

	Anaerobes	Brucella	Gen. Purpose Culture Media	Gram (-) Enteric Bacteria	Pseudomonas	Salmonella/Shigella	Staphylococcus	Streptococcus	Yeast and Mold	Mycobacteria	Campylobacter
Pseudomonas F Agar (7312)				X							
Pseudomonas Isolation Agar (7329)				X							
Pseudomonas Isolation Broth (7474)				X							
Pseudomonas P Agar (7328)				X							
Sabouraud BHI Agar (7235)								X			
Sabouraud Dextrose Agar (7150)								X			
Sab-Dex Agar w/ Chloramp (7306)								X			
Salmonella Shigella Agar (7152)					X						
Schaedler Agar (7153)	X										
Schaedler Broth (7154)	X										
Selective Strep Agar, Mod. No. 2 (7405)							X				
Selenite Broth (7155)					X						
Selenite Cystine Broth (7283)					X						
Soy Peptone Yeast Extract (7224)								X			
Standard Methods Agar (7157)											
Staph Selective Agar (7373)						X					
Staphylococcus Agar No. 110 (7158)						X					
Tetrathionate Broth Base (7241)					X						
Tryptic Soy Agar (7100)			X								
Tryptic Soy Broth (7164)			X								
Tryptose Agar (7347)		X									
Vogel & Johnson Agar (7207)						X					
XL Agar Base (7223)				X							
XLD Agar (7166)					X						
XLT4 Agar (7517)					X						
YM Agar (7525)								X			

Market-Specific Information

Cosmetics

	Environmental	Gram (-) Screening	Pseudomonas Isolation	Staphylococcus	Sterility Testing	Yeast and Mold Isolation
Cetrimide Agar Base (7222)			X			
D/E Neutralizing Agar (7375)	X					
D/E Neutralizing Broth (7562)	X					
Eosin Methylene Blue Agar (7134)		X				
Fluid Thioglycollate Medium (7137)					X	
HC Agar Base (7520)						X
Lethen Agar Base (7118)	X					
Lethen Agar Base, Modified (7495)	X					
Lethen Broth Base (7105)	X					
Lethen Broth Base, Modified (7496)	X					
MacConkey Agar (7102)		X				
Malt Agar (7456)						X
Malt Extract Agar (7303)						X
Mannitol Salt Agar (7143)				X		
Mycobiotic Agar (7419)						X
Mycological Agar (7309)						X
Phenylethanol Agar (7147)				X		
Potato Dextrose Agar (7149)						X
Pseudomonas F Agar (7312)			X			
Pseudomonas Isolation Broth (7474)			X			
Pseudomonas P Agar (7328)			X			
Sabouraud Dextrose Agar (7150)						X
Staphylococcus Agar No. 110 (7158)				X		
TAT Broth (7219)					X	
TSA w/ Lecithin & Tween 80 (7163)						
Tryptic Soy Broth (7164)					X	
Vogel & Johnson Agar (7207)				X		

Market-Specific Information

Dairy

	Coliforms Analysis	Environmental	Listeria testing	Standard Plate Count	Streptococcal Detection	Yeast and Mold Isolation
Azide Dextrose Broth (7315)				X		
Brilliant Green Bile Broth 2% (7119)	X					
D/E Neutralizing Agar (7375)		X				
D/E Neutralizing Broth (7562)		X				
EC Medium w/ MUG (7361)	X					
Eosin Methylene Blue, Levine (7103)	X					
Fraser Broth (7626)			X			
Fraser Broth Base (7502)			X			
LPM Agar (7424)			X			
Lactose Broth (7141)	X					
Lauryl Sulfate Broth w/ MUG (7300)	X					
Lethen Agar Base (7118)		X				
Lethen Broth Base (7105)		X				
M17 Broth Base (7450)				X		
mFC Agar (7397)	X					
Malt Extract Agar (7303)						X
Motility Test Agar (7247)			X			
Nutrient Agar (7145)		X				
Nutrient Broth (7146)	X					
Oxford Listeria Agar (7428)			X			
Phenylethanol Agar (7147)				X		
Potato Dextrose Agar (7149)						X
Sabouraud Dextrose Agar (7150)						X
Standard Methods Agar (7157)				X		
Tryptic Soy Broth (7164)		X				
TSA w/ Lecithin & Tween 80 (7163)		X				
UVM Mod. Listeria Enrichment Broth (7409)			X			
Universal Pre-enrichment Broth (7510)			X			
Violet Red Bile Agar (7165)	X					
Violet Red Bile Agar w/ MUG (7359)	X					

Market-Specific Information

Food and Beverage

	Bacillus	Beverage Analysis	Campylobacter	Clostridium	Coliform Analysis	Environmental	Lactobacillus	Listeria	Pseudomonas	Salmonella/Shigella	Staphylococcus	Total Plate Count	Yeast and Mold	Vibrio	Yersinia
A-1 Medium (7601)			X												
APT Agar (7302)						X									
Bacillus Cereus Agar Base (7442)	X														
Baird Parker Agar (7112)										X					
Bismuth Sulfite Agar (7113)									X						
Brilliant Green Agar (7117)									X						
Brilliant Green Agar w/ Sulfadiazine (7310)									X						
Brilliant Green Agar w/ Sulfapyridine (7299)									X						
Brilliant Green Bile Agar (7449)				X											
Brilliant Green Bile Broth 2% (7119)				X											
Brilliant Green Bile Broth 2% w/ MUG (7344)				X											
Brucella Agar (7120)			X												
Brucella Broth (7121)			X												
Buffered Listeria Enrichment Broth (7579)							X								
Buffered Peptone Water (7418)									X						
Campylobacter Selective Agar Base (7443)			X												
Campy Blood Free Selective Medium (7527)			X												
Campylobacter Enrichment Broth (7526)			X												
Cetrimide Agar (7222)								X							
Clostridium Difficile Agar (7385)				X											
Cooked Meat Medium (7110)				X											
D/E Neutralizing Agar (7375)					X										
D/E Neutralizing Broth (7562)					X										
Dextrose Tryptone Agar (7340)	X														
Dextrose Tryptone Broth (7338)	X														
Dichloran Glycerol (DG-18) Ag Base (7592)												X			
DNase Test Agar (7129)										X					
DNase Test Agar w/ Methyl Green (7260)										X					
DRBC Agar (7591)												X			
EC Medium w/ MUG (7361)				X											
EC Medium, Modified (7506)				X											
EC Medium (7206)				X											
EMB, Levine (7103)				X											
Fluid Thioglycollate Medium (7137)			X												
Fraser Broth (7626)							X								
Fraser Broth Base (7502)							X								
Hektoen Enteric Agar (7138)									X						
Lactobacilli MRS Agar (7543)						X									
Lactobacilli MRS Broth (7406)						X									
Lactobacillus Selective Agar Base (7234)						X									
Lactose Broth (7141)									X						
Lauryl Sulfate Broth (7142)				X											
Lauryl Sulfate Broth w/ MUG (7300)				X											
Lethen Agar Base (7118)					X										
Lethen Broth Base (7105)					X										
Listeria Enrichment Broth (7398)							X								
LPM Agar (7424)							X								
M-Broth (7296)									X						

Food and Beverage (cont.)

	Bacillus	Beverage Analysis	Campylobacter	Clostridium	Coliform Analysis	Environmental	Lactobacillus	Listeria	Pseudomonas	Salmonella/Shigella	Staphylococcus	Total Plate Count	Yeast and Mold	Vibrio	Yersinia
MacConkey Agar (7102)				X											
MacConkey Agar w/Sorbitol (7320)				X											
MacConkey Agar, CS (7391)				X											
MacConkey Broth (7185)				X											
Malt Agar (7456)												X			
Malt Extract Agar (7303)												X			
Mannitol Salt Agar (7143)										X					
Motility Test Agar (7247)							X								
Mycological Agar (7309)												X			
Orange Serum Agar (7587)		X													
Oxford Listeria Agar (7428)							X								
Potato Dextrose Agar (7149)												X			
PDA w/ Lecithin & Tween 80 (7575)					X							X			
Potato Dextrose Broth (7585)												X			
Pseudomonas F Agar (7312)								X							
Pseudomonas Isolation Agar (7329)								X							
Pseudomonas P Agar (7328)								X							
Rappaport-Vassiliadis, MSRV (7511)									X						
Rappaport-Vassiliadis R10 Broth (7512)									X						
Sabouraud Dextrose Agar (7150)												X			
Sab-Dex w/ Lecithin & Tween 80 (7392)					X							X			
Salmonella Shigella Agar (7152)									X						
Selenite Broth (7155)									X						
Selenite Cystine Broth (7283)									X						
Standard Methods Agar (7157)											X				
Staph Selective Agar (7373)										X					
Staphylococcus Agar No. 110 (7158)										X					
TCBS Agar (7210)													X		
Tergitol 7 Agar (7187)				X											
Tetrathionate Broth Base (7241)									X						
Tomato Juice Agar (7349)						X									
Tryptic Soy Agar (7100)											X				
Tryptic Soy Agar w/ Lec & Tween 80 (7163)					X										
Tryptic Soy Broth (7164)					X										
Universal Beer Agar (7574)		X													
Universal Pre-enrichment Broth (7510)							X								
UVM Modified Listeria Enrich Broth (7409)							X								
Violet Red Bile Agar (7165)				X											
Violet Red Bile Agar w/ MUG (7359)				X											
Violet Red Bile Glucose Agar (7425)				X											
Vogel & Johnson Agar (7207)										X					
W-L Nutrient Medium (7488)		X													
XLD Agar (7166)									X						
XLT4 Agar Base (7517)									X						
Yersinia Selective Agar (7257)														X	
YM Agar (7525)												X			
YM Broth (7363)												X			

Market-Specific Information

Veterinary

	Brucella	Clostridium	Gen. Purpose Culture Media	Gram (-) Enteric Bacteria	Pseudomonas	Salmonella/Shigella	Staphylococcus	Streptococcus	Yeast and Mold
Baird Parker Agar (7112)						X			
Beta-SSA Agar (7336)							X		
BIGGY Agar (7191)									X
Bile Esculin Agar (7249)							X		
Bile Esculin Azide Agar (7133)							X		
Bismuth Sulfite Agar (7113)					X				
Blood Agar Base No. 2 (7266)			X						
Brain-Heart Infusion Agar (7115)			X						X
Brilliant Green Bile Agar (7449)					X				
Brucella Agar (7120)	X								
Brucella Broth (7121)	X								
Cetrimide Agar (7222)				X					
Cooked Meat Medium (7110)		X							
Columbia Blood Agar Base (7125)			X						
Deoxycholate Agar (7130)				X					
Dermatophyte Test Medium (7265)									X
Eosin Methylene Blue Agar (7134)				X					
EMB, Levine (7103)				X					
Eugonic Agar (7135)	X								
Fluid Thioglycollate Medium (7137)		X	X						
Fungisel Agar (7205)									X
Heart Infusion Agar (7269)			X						
Hektoen Enteric Agar (7138)					X				
Inhibitory Mold Agar (7238)									X
MacConkey Agar (7102)				X					
MacConkey Agar, CS (7391)				X					
Malt Agar (7456)									X
Malt Extract Agar (7303)									X
Mannitol Salt Agar (7143)						X			
MRSA Agar Base (7420)						X			
Mycobiotic Agar (7419)									X
Mycological Agar (7309)									X
Nutrient Agar (7145)			X						
Phenylethanol Agar (7147)						X	X		
Potato Dextrose Agar (7149)									X
Potato Infusion Agar (7532)	X								
Pseudomonas F Agar (7312)				X					
Pseudomonas Isolation Agar (7329)				X					
Pseudomonas Isolation Broth (7474)				X					
Pseudomonas P Agar (7328)				X					
Sabouraud BHI Agar (7235)									X
Sabouraud Dextrose Agar (7150)									X
Sab-Dex Agar w/ Chloramp (7306)									X

Veterinary (cont.)

	<i>Brucella</i>	<i>Clostridium</i>	Gen. Purpose Culture Media	Gram (-) Enteric Bacteria	<i>Pseudomonas</i>	<i>Salmonella/Shigella</i>	<i>Staphylococcus</i>	<i>Streptococcus</i>	Yeast and Mold
Salmonella Shigella Agar (7152)					X				
Schaedler Agar (7153)		X							
Schaedler Broth (7154)		X							
Selective Strep Agar, Mod No. 2 (7405)							X		
Selenite Broth (7155)					X				
Selenite Cystine Broth (7283)					X				
Soy Peptone Yeast Extract (7224)									X
Standard Methods Agar (7157)									
Staph Selective Agar (7373)						X			
Staphylococcus Agar #110 (7158)						X			
Tetrathionate Broth Base (7241)					X				
Tryptic Soy Agar (7100)			X						
Tryptic Soy Broth (7164)			X						
Tryptose Agar (7347)	X								
Tryptose Broth (7367)	X								
Vogel & Johnson Agar (7207)						X			
XL Agar Base (7223)				X					
XLD Agar (7166)				X					
XLT4 Agar (7517)					X				
YM Agar (7525)									X
YM Broth (7363)									X

Market-Specific Information

Water and Wastewater

	Standard Plate Count	Total Coliforms	Fecal Coliforms	Fecal Streptococcus	Salmonella spp.	Stressed Organisms	Clostridium perfringens	Legionella spp.	Pseudomonas spp.
A-1 Medium (7601)			X						
Azide Dextrose Broth (7315)				X					
BCYE Agar (Legionella Agar) (7307)								X	
Bile Esculin Agar (7249)				X					
Bile Esculin Azide Agar (7133)				X					
Bismuth Sulfite Agar (7113)					X				
Brilliant Green Agar (7117)					X				
Brilliant Green Bile Agar (7449)		X							
Brilliant Green Bile Broth 2% (7119)		X							
Cetrimide Agar Base (7222)									X
EC Medium (7206)			X						
EC Medium w/MUG (7361)			X						
Eosin Methylene Blue, Levine (7103)			X						
Lauryl Sulfate Broth (7142)			X						
Lauryl Sulfate Broth w/MUG (7300)			X						
MCP Agar (7477)							X		
m-FC Agar (7397)			X						
m-FC Broth (7396)			X						
m-Enterococcus Agar (7544)			X	X					
m-TEC Agar (7421)						X			
MacConkey Agar, Modified (7440)			X						
Presence-Absence Broth (7500)		X							
Pseudomonas F Agar (7312)									X
Pseudomonas Isolation Agar (7329)									X
Pseudomonas Isolation Broth (7474)									X
Pseudomonas P Agar (7328)									X
R2A Agar (7390)						X			
Selenite Broth (7155)					X				
Selenite Cystine Broth (7283)					X				
Standard Methods Agar (7157)	X								
Tetrathionate Broth Base (7241)					X				
XLD Agar (7166)					X				

Microorganism-Specific Information

E. coli

	Nonselective Media	Purity Plate	Selective-Differential	<i>E. coli</i> O157:H7
Brain Heart Infusion Agar (7115)	X			
Brilliant Green Bile Agar (7449)			X	
EC Medium w/MUG (7361)			X	
EC Medium, Modified (7506)				X
Eosin Methylene Blue Agar (7134)			X	
Lauryl Sulfate Broth w/MUG (7300)			X	
MacConkey Agar (7102)			X	
MacConkey Agar w/Sorbitol (7320)				X
Nutrient Agar (7145)	X			
Tryptic Soy Agar (7100)	X	X		
Violet Red Bile Agar (7165)			X	
Violet Red Bile Agar w/MUG (7359)			X	

Coliforms

	Food Analysis	Environmental
A-1 Medium (7601)		X
Brilliant Green Bile Agar (7449)	X	X
Brilliant Green Bile Broth 2% (7119)	X	X
Brilliant Green Bile Broth 2% w/MUG (7344)	X	X
EC Medium (7206)	X	X
EC Medium, Modified (7506)	X	
EC Medium w/ MUG (7361)	X	X
Eosin Methylene Blue Agar, Levine (7103)	X	X
Lactose Broth (7141)	X	X
Lauryl Sulfate Broth (7142)		X
Lauryl Sulfate Broth w/MUG (7300)		X
MacConkey Agar (7102)	X	X
MacConkey Agar w/ Sorbitol (7320)	X	X
MacConkey Agar, CS (7391)	X	X
MacConkey Agar, Modified (7440)	X	
MacConkey Broth (7185)	X	X
Tergitol 7 Agar (7187)	X	X
Violet Red Bile Agar (7165)	X	X
Violet Red Bile Agar w/ MUG (7359)	X	X
Violet Red Bile Glucose Agar (7425)	X	

Microorganism-Specific Information

Salmonella

	Differential Agar	Enrichment Media	Highly Selective Media	Differential (Identification) Media
Bismuth Sulfite Agar (7113)			X	
Brilliant Green Agar (7117)			X	
Brilliant Green Agar w/ Sulfadiazine (7310)			X	
Brilliant Green Agar w/ Sulfapyridine (7299)			X	
Buffered Peptone Water (7418)		X		
Deoxycholate Agar (7103)	X			
Deoxycholate Citrate Agar (7186)	X			
Eosin Methylene Blue Agar (7134)	X			
Eosin Methylene Blue, Levine (7103)	X			
GN Broth (Hajna) (7218)		X		
Hektoen Enteric Agar (7138)	X			
Lysine Iron Agar (7211)				X
M-Broth (7296)		X		
MacConkey Agar (7102)	X			
MacConkey Agar, CS (7391)	X			
MIO Medium (7389)				X
Rappaport-Vassiliadis (MSRV) Medium (7511)			X	
Rappaport-Vassiliadis R10 Broth (7512)		X		
Salmonella Shigella Agar (7152)			X	
Selenite Broth (7155)		X		
Selenite Cystine Broth (7283)		X		
SIM Medium (7221)				X
Simmons Citrate Agar (7156)				X
Tetrathionate Broth Base (7241)		X		
Triple Sugar Iron (7162)				X
Universal Pre-enrichment Broth (7510)		X		
XL Agar Base (7223)	X			
XLD Agar (7166)	X			
XLT4 Agar (7517)	X			

Listeria

	Differential Agar	Enrichment Media
Buffered Listeria Enrichment Broth (7579)		X
Fraser Broth (7626)		X
Fraser Broth Base (7502)		X
Listeria Enrichment Broth (7398)		X
LPM Agar (7424)	X	
Motility Test Agar (7247)	X	
Oxford Listeria Agar (7428)	X	
Universal Pre-enrichment Broth (7510)		X

Microorganism-Specific Information

Campylobacter

	Enrichment Agar	Enrichment Broth	Highly Selective Media
Brucella Agar (7120)	X		
Campy Blood Free Selective Med.(CCDA) (7527)			X
Campy Selective Agar Base (Preston) (7443)			X
Campylobacter Enrichment Broth (7526)		X	

Yeast and Mold

	Nonselective Media	Enrichment Media	Selective Media
Dextrose Tryptone Broth (7338)		X	
Dichloran Glycerol (DG-18) Agar Base (7592)			X
DRBC Agar (7591)			X
Malt Agar (7456)	X		
Malt Extract Agar (7303)	X		
Mycobiotic Agar (7419)			X
Mycological Agar (7309)	X		
Orange Serum Agar (7587)		X	
Potato Dextrose Agar (7149)	X		
Potato Dextrose Agar w/Lecithin & Tween 80 (7575)	X		
Potato Dextrose Broth (7585)		X	
Sabouraud Dextrose Agar (7150)	X		
Sabouraud Dextrose Agar w/Lecithin & Tween 80 (7392)	X		
Universal Beer Agar (7574)		X	
W-L Nutrient Medium (7488)	X		
YM Agar (7525)	X		
YM Broth (7363)	X		

Total Plate Count

	Food Analysis	Environmental
Blood Agar Base No. 2 (7266)	X	
Blood Agar Base, Improved (7268)	X	
Blood Agar Base w/Low pH (7354)	X	
Blood Agar Base, pH 7.4 (7114)	X	
Brain-Heart Infusion Agar (7115)		X
Brucella Agar (7120)	X	
Columbia Blood Agar Base (7125)	X	
Heart Infusion Agar (7269)	X	X
Nutrient Agar (7145)	X	X
Standard Methods Agar (7157)	X	X
Tryptic Soy Agar (7100)	X	X
Tryptose Agar (7347)	X	
Tryptose Blood Agar Base (7282)	X	

ISO-GRID™ Dehydrated Culture Media

ISO-GRID dehydrated culture media is used in conjunction with the ISO-GRID Membrane Filtration System. This system is based on the principle of hydrophobic grid membrane filtration. Target organisms are detected and enumerated through the use of a unique membrane filter. The ISO-GRID membrane filter is subdivided into a 40 x 40 hydrophobic grid matrix, surrounded by a hydrophobic border.

Prepared sample homogenates are first passed through a 5 µm stainless steel prefilter to eliminate food particles that could interfere with the microbial analysis. Next, samples are filtered through the hydrophobic membrane. The membrane is then placed on a specific ISO-GRID culture medium formulated for optimum organism growth. Dyes and selective agents are incorporated into ISO-GRID culture media to detect and differentiate target organisms.

After a specified incubation period, growth on the membrane filter is examined and enumerated. Enumeration is calculated by counting the number of squares containing the target organism. The total number of positive squares is converted to the corresponding most probable number (MPN) using one of the methods described in the ISO-GRID Methods Manual.

For complete information concerning any ISO-GRID method, refer to the ISO-GRID Methods Manual.

The following culture media are used in ISO-GRID Filtration System Methods:

• BMA Agar, for confirmation of <i>E. coli</i>	41
• EF-18 Agar, for <i>Salmonella</i> detection	43
• LM-137 Agar, for <i>Listeria</i> detection	45
• LMG Agar, for <i>E. coli</i> / coliform enumeration	47
• SCCRAM Broth, <i>Salmonella</i> enrichment broth	49
• SD-39 Agar, for enumeration of <i>E. coli</i> / <i>E. coli</i> O157:H7	51
• TSAF Agar, for total bacterial count	53
• YM-11 Agar, for enumeration of yeasts and molds	55

Acumedia Dehydrated Culture Media

Listed in alphabetical order

A-1 Medium	Dermatophyte Test Medium
Agar, Bacteriological	Dextrose Tryptone Agar
APT Agar	Dextrose Tryptone Broth
Azide Dextrose Broth	Dichloran Glycerol (DG-18) Agar Base
Bacillus Cereus Agar Base	Dipeptone
Baird Parker Agar	DNase Test Agar
BCYE Agar (Legionella Agar)	DNase Test Agar w/ Methyl Green
Beef Extract Powder	DRBC Agar
Beta-SSA Agar	EC Medium
BIGGY Agar	EC Medium w/ MUG
Bile Esculin Agar	EC Medium, Modified
Bile Esculin Azide Agar	Elliker Broth
Bile Salts Mixture # 3	EMB Agar (Holt, Harris & Teague)
Bismuth Sulfite Agar	EMB Agar, Levine
Blood Agar Base No.2	Eugonic Agar
Blood Agar Base w/ Low pH	Fastidious Anaerobe Agar
Blood Agar Base, Improved	Fluid Thioglycollate Medium
Blood Agar Base, pH 7.4	Fraser Broth
Brain-Heart Infusion Agar	Fraser Broth Base
Brain-Heart Infusion Broth	Fungisel Agar
Brain-Heart Infusion Solids	GC Agar
Brain-Heart Infusion w/o Dextrose	Gelatin
Brilliant Green Agar	GN Broth (Hajna)
Brilliant Green Agar w/ Sulfadiazine	HC Agar Base
Brilliant Green Agar w/ Sulfapyridine	Heart Infusion Agar
Brilliant Green Bile Agar	Heart Infusion Broth
Brilliant Green Bile Broth 2%	Hektoen Enteric Agar
Brilliant Green Bile Broth 2% w/ MUG	Hemoglobin Powder
Brucella Agar	Inhibitory Mold Agar
Brucella Broth	Kligler Iron Agar
Buffered Listeria Enrichment Broth	Lactobacilli MRS Agar
Buffered Peptone Water	Lactobacilli MRS Broth
Campy Blood Free Selective Medium (CCDA)	Lactobacillus Selective Agar Base
Campy Selective Agar Base (Preston)	Lactose Broth
Campylobacter Enrichment Broth	Lauryl Sulfate Broth
Cary and Blair Transport Medium	Lauryl Sulfate Broth w/ MUG
Cary and Blair Trans Med, Mod w/ Phenol Red	LB Agar (Lennox L Agar)
Casein, Acid Hydrolysate	LB Broth (Lennox L Broth)
Casman Medium Base	Lethen Agar Base
CDC Anaerobe Agar	Lethen Agar Base, Modified
Cetrimide Agar	Lethen Broth Base
Charcoal Agar	Lethen Broth Base, Modified
CHO Medium Base	Listeria Enrichment Broth
CLED Agar	Littman Agar
Clostridium Difficile Agar	Lowenstein-Jensen Medium
Columbia Blood Agar Base	LPM Agar
Columbia Broth	Luria Agar (Miller's LB Agar)
Columbia CNA Agar	Luria Broth Base (Miller's LB Broth)
Cooked Meat Medium	Lysine Iron Agar
D/E Neutralizing Agar	M-Broth
D/E Neutralizing Broth	m-Enterococcus Agar
Deoxycholate Agar	m-FC Agar
Deoxycholate Citrate Agar	m-FC Broth

m-Green Yeast and Fungi Broth	Sabouraud BHI Agar
m-TEC Agar	Sabouraud Dextrose Agar
m-TGE Broth	Sabouraud Dextrose Agar w/ Chloramphenicol
M17 Broth Base	Sabouraud Dextrose Agar w/ Lec & Tween 80
MacConkey Agar	Sabouraud Dextrose Agar, Emmons
MacConkey Agar w/o Crystal Violet	Salmonella Shigella Agar
MacConkey Agar w/o Crystal Violet & Salt	Schaedler Agar
MacConkey Agar w/ Sorbitol	Schaedler Broth
MacConkey Agar, CS	Selective Strep Agar, Modified #2
MacConkey Agar, Modified	Selenite Broth
MacConkey Broth	Selenite Cystine Broth
Malonate Broth	SIM Medium
Malt Agar	Simmons Citrate Agar
Malt Extract	Skim Milk
Malt Extract Agar	Soy Peptone Yeast Extract Agar
Mannitol Salt Agar	Standard Methods Agar
MCP Agar	Staph Selective Agar
Middlebrook 7H10 Agar	Staphylococcus Agar # 110
Middlebrook 7H11 Agar	Sterility Test Broth
MIO Medium	TAT Broth
Mitis Salivarius Agar	TCBS Agar
Motility Test Agar	Tergitol 7 Agar
MRSA Agar Base	Tetrathionate Broth Base
MR-VP Broth	Thioglycollate Medium w/o Indicator
Mueller Hinton Agar	Todd Hewitt Broth
Mycobiotic Agar	Tomato Juice Agar
Mycological Agar	Triple Sugar Iron Agar
Nutrient Agar	Tryptic Soy Agar
Nutrient Agar 1.5%	Tryptic Soy Agar w/ Lec & Tween 80
Nutrient Broth	Tryptic Soy Broth
Nutrient Gelatin	Tryptone
Orange Serum Agar	Tryptone Glucose Extract Agar
Ox bile (Ox gall)	Tryptose Agar
Oxford Listeria Agar Base	Tryptose Blood Agar Base
Pancreatic Digest of Casein (Peptone C)	Tryptose Broth
Pancreatic Digest of Gelatin (Peptone G)	Tryptose Phosphate Broth
Papaic Digest of Soybean Meal (Peptone S)	Universal Beer Agar
Peptic Digest of Animal Tissue (Peptone A)	Universal Pre-Enrichment Broth
Peptone Water	Urea Agar Base
Phenol Red Broth Base	UVM Mod Listeria Enrichment Broth
Phenylethanol Agar	Violet Red Bile Agar
Phosphate Buffer, pH 7.2	Violet Red Bile Agar w/ MUG
Potato Dextrose Agar	Violet Red Bile Glucose Agar
Potato Dextrose Agar with Lec & Tween 80	Vogel and Johnson Agar
Potato Dextrose Broth	Wilkins-Chalgren Agar
Potato Infusion Agar	Wilkins-Chalgren Broth
Presence-Absence Broth	W-L Nutrient Medium
Pseudomonas F Agar	XL Agar Base
Pseudomonas Isolation Agar	XLD Agar
Pseudomonas Isolation Broth	XLT4 Agar
Pseudomonas P Agar	Yeast Enriched Peptone
Purple Lactose Agar	Yeast Extract
R2A Agar	Yersinia Selective Agar
Rappaport-Vassiliadis (MSRV) Medium	YM Agar
Semisolid Modified	YM Broth
Rappaport-Vassiliadis R10 Broth	



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Acumedia Product Information Sheets



Acumedia Dehydrated Culture Media

Listed in alphabetical order

A-1 Medium	Dermatophyte Test Medium
Agar, Bacteriological	Dextrose Tryptone Agar
APT Agar	Dextrose Tryptone Broth
Azide Dextrose Broth	Dichloran Glycerol (DG-18) Agar Base
Bacillus Cereus Agar Base	Dipeptone
Baird Parker Agar	DNase Test Agar
BCYE Agar (Legionella Agar)	DNase Test Agar w/ Methyl Green
Beef Extract Powder	DRBC Agar
Beta-SSA Agar	EC Medium
BIGGY Agar	EC Medium w/ MUG
Bile Esculin Agar	EC Medium, Modified
Bile Esculin Azide Agar	Elliker Broth
Bile Salts Mixture # 3	EMB Agar (Holt, Harris & Teague)
Bismuth Sulfite Agar	EMB Agar, Levine
Blood Agar Base No.2	Eugonic Agar
Blood Agar Base w/ Low pH	Fastidious Anaerobe Agar
Blood Agar Base, Improved	Fluid Thioglycollate Medium
Blood Agar Base, pH 7.4	Fraser Broth
Brain-Heart Infusion Agar	Fraser Broth Base
Brain-Heart Infusion Broth	Fungisel Agar
Brain-Heart Infusion Solids	GC Agar
Brain-Heart Infusion w/o Dextrose	Gelatin
Brilliant Green Agar	GN Broth (Hajna)
Brilliant Green Agar w/ Sulfadiazine	HC Agar Base
Brilliant Green Agar w/ Sulfapyridine	Heart Infusion Agar
Brilliant Green Bile Agar	Heart Infusion Broth
Brilliant Green Bile Broth 2%	Hektoen Enteric Agar
Brilliant Green Bile Broth 2% w/ MUG	Hemoglobin Powder
Brucella Agar	Inhibitory Mold Agar
Brucella Broth	Kligler Iron Agar
Buffered Listeria Enrichment Broth	Lactobacilli MRS Agar
Buffered Peptone Water	Lactobacilli MRS Broth
Campy Blood Free Selective Medium (CCDA)	Lactobacillus Selective Agar Base
Campy Selective Agar Base (Preston)	Lactose Broth
Campylobacter Enrichment Broth	Lauryl Sulfate Broth
Cary and Blair Transport Medium	Lauryl Sulfate Broth w/ MUG
Cary and Blair Trans Med, Mod w/ Phenol Red	LB Agar (Lennox L Agar)
Casein, Acid Hydrolysate	LB Broth (Lennox L Broth)
Casman Medium Base	Lethen Agar Base
CDC Anaerobe Agar	Lethen Agar Base, Modified
Cetrimide Agar	Lethen Broth Base
Charcoal Agar	Lethen Broth Base, Modified
CHO Medium Base	Listeria Enrichment Broth
CLED Agar	Littman Agar
Clostridium Difficile Agar	Lowenstein-Jensen Medium
Columbia Blood Agar Base	LPM Agar
Columbia Broth	Luria Agar (Miller's LB Agar)
Columbia CNA Agar	Luria Broth Base (Miller's LB Broth)
Cooked Meat Medium	Lysine Iron Agar
D/E Neutralizing Agar	M-Broth
D/E Neutralizing Broth	m-Enterococcus Agar
Deoxycholate Agar	m-FC Agar
Deoxycholate Citrate Agar	m-FC Broth

m-Green Yeast and Fungi Broth	Sabouraud BHI Agar
m-TEC Agar	Sabouraud Dextrose Agar
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Malonate Broth	SIM Medium
Malt Agar	Simmons Citrate Agar
Malt Extract	Skim Milk
Malt Extract Agar	Soy Peptone Yeast Extract Agar
Mannitol Salt Agar	Standard Methods Agar
MCP Agar	Staph Selective Agar
Middlebrook 7H10 Agar	Staphylococcus Agar # 110
Middlebrook 7H11 Agar	Sterility Test Broth
MIO Medium	TAT Broth
Mitis Salivarius Agar	TCBS Agar
Motility Test Agar	Tergitol 7 Agar
MRSA Agar Base	Tetrathionate Broth Base
MR-VP Broth	Thioglycollate Medium w/o Indicator
Mueller Hinton Agar	Todd Hewitt Broth
Mycobiotic Agar	Tomato Juice Agar
Mycological Agar	Triple Sugar Iron Agar
Nutrient Agar	Tryptic Soy Agar
Nutrient Agar 1.5%	Tryptic Soy Agar w/ Lec & Tween 80
Nutrient Broth	Tryptic Soy Broth
Nutrient Gelatin	Tryptone
Orange Serum Agar	Tryptone Glucose Extract Agar
Oxbile (Oxgall)	Tryptose Agar
Oxford Listeria Agar Base	Tryptose Blood Agar Base
Pancreatic Digest of Casein (Peptone C)	Tryptose Broth
Pancreatic Digest of Gelatin (Peptone G)	Tryptose Phosphate Broth
Papaic Digest of Soybean Meal (Peptone S)	Universal Beer Agar
Peptic Digest of Animal Tissue (Peptone A)	Universal Pre-Enrichment Broth
Peptone Water	Urea Agar Base
Phenol Red Broth Base	UVM Mod Listeria Enrichment Broth
Phenylethanol Agar	Violet Red Bile Agar
Phosphate Buffer, pH 7.2	Violet Red Bile Agar w/ MUG
Potato Dextrose Agar	Violet Red Bile Glucose Agar
Potato Dextrose Agar with Lec & Tween 80	Vogel and Johnson Agar
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Pseudomonas F Agar	XL Agar Base
Pseudomonas Isolation Agar	XLD Agar
Pseudomonas Isolation Broth	XLT4 Agar
Pseudomonas P Agar	Yeast Enriched Peptone
Purple Lactose Agar	Yeast Extract
R2A Agar	Yersinia Selective Agar
Rappaport-Vassiliadis (MSRV) Medium	YM Agar
Semisolid Modified	YM Broth
Rappaport-Vassiliadis R10 Broth	

A-1 MEDIUM (7601)

Intended Use

A-1 Medium is used for the detection of coliform organisms in water and food.

Product Summary and Explanation

Since the early 1900's enumeration of coliform organisms, specifically *E. coli*, has been used to determine water purity. Elevated temperature and most-probable-number (MPN) methods are routinely used for analysis of water and food samples for presence of fecal coliforms. A-1 Medium was formulated to hasten *E. coli* recovery and reduce the incidence of false positive cultures.

In 1972 Andrews and Presnell developed A-1 Medium.¹ This medium recovers *E. coli* from estuarine water in 24 hours instead of 72 hours, and in greater numbers without the pre-enrichment step.² 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. A-1 Medium conforms to standard methods for the isolation of fecal coliforms in water and foods.³⁻⁵

Principles of the Procedure

Enzymatic Digest of Casein provides sources of nitrogen, amino acids, and vitamins 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.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	20 g
Lactose	5 g
Sodium Chloride	5 g
Salicin	0.5 g
Triton X-100	0.5 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 31.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Distribute into test tubes containing Durham tubes and autoclave at 121°C for 10 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, not free flowing and light beige.

Prepared Appearance: Prepared medium is very light amber and clear (flocculent precipitate may be present at room temperature).

Expected Cultural Response: Cultural response in A-1 Medium after 3 hours at 35°C and subsequent incubation for 21 – 22 hours at 44.5°C.

Microorganism	Response	Reactions (Gas)
<i>Escherichia coli</i> ATCC® 25922	growth	positive
<i>Escherichia coli</i> ATCC® 35218	growth	positive
<i>Proteus mirabilis</i> ATCC® 12453	growth	negative
<i>Pseudomonas aeruginosa</i> ATCC® 27853	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate tubes of A-1 Medium as directed in standard methods.³⁻⁵
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.

Results

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.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if appearance has changed from the original color and texture. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. Fecal coliform counts are usually greater than *E. coli* counts.⁴
3. Interpretation of test procedure using A-1 Medium requires understanding of the microflora of the specimen.⁴

Packaging

A-1 Medium	Code No.	7601A	500 g
		7601B	2 kg
		7601C	10 kg

References

1. **Andrews, W. H., and M. W. Presnell.** 1972. Rapid recovery of *Escherichia coli* from estuarine water. Appl. Microbiol. **23**:521-523.
2. **Andrews, W. H., C. D. Diggs, and C. R. Wilson.** 1975. Evaluation of a medium for the rapid recovery of *Escherichia coli* from shellfish. Appl. Microbiol. **29**:130-131.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, M.D.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

AGAR, Bacteriological (7178)

Intended Use

Agar, Bacteriological is a solidifying agent for use in preparing microbiological culture media.

Product Summary and Explanation

Agar is a phycocolloid extracted from a group of red-purple algae, usually *Gelidium* spp. Agar was first suggested for microbiological purposes in 1881 by Fannie Hesse.^{1,2} By the early 1900's, agar became the gelling agent of choice over gelatin because agar remains firm at growth temperature for many pathogens and Agar is 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 to study pure cultures.

Agar is a gel at room temperature, remaining firm at temperatures as high as 65°C.³ Agar melts at approximately 85-91°C, a different temperature from solidification at 34-36°C. This property is known as hysteresis. Agar is generally resistant to shear forces; however, different agar may have different gel strengths or degrees of stiffness.

Specifications for Agar, Bacteriological 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. Agar, Bacteriological is recommended for clinical applications, auxotrophic studies, bacterial and yeast transformation studies, and bacterial molecular genetics applications.^{4,5}

Principles of the Procedure

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), growth of anaerobes (0.1%) and microaerophiles.³

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is granular, homogeneous, free-flowing and creamy white beige.

Prepared Appearance (1.5% wt/vol): Prepared medium is very light amber to medium amber and slightly opalescent.

pH (2% Solution at 25°C): 6.0 - 7.5

Gel Strength: 760 - 850 g/cm²

Expected Cultural Response: Cultural response in Peptone Agar after incubation at 35°C for 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to good growth

Test Procedure

Refer to appropriate references for specific procedures using Agar, Bacteriological.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Agar, Bacteriological at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Agar, Bacteriological should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Agar, Bacteriological in its intact container when stored as directed.

Packaging

Agar, Bacteriological	Code No.	7178A	500 g
		7178B	2 kg
		7178C	10 kg

References

1. **Hesse, W.** 1894. Über die quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen. Mit. a.d. Kaiserl. Gesh. Berlin. **2**: 182-207.
2. **Hitchens, A. P., and M. C. Leiking.** 1939. The introduction of agar-agar into bacteriology. J. Bacteriol. **37**:485-493.
3. **Selby, H. H., and T. A. Selby.** 1959. Agar. In Whister (ed.). Industrial gums, Academic Press Inc., New York, N. Y.
4. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning, a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York, N. Y.
5. **Schiestl, R. H., and R. Daniel Geitz.** 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Current Genetics. **16**:339-346.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

APT AGAR (7302)

Intended Use

APT Agar is used for the cultivation of heterofermentative lactobacilli.

Product Summary and Explanation

Evans and Niven investigated the cultivation of heterofermentative lactobacilli, causing the faded or green discoloration of cured meat products.¹ Deibel, Evans, and Niven tested thiamine-requiring bacteria, specifically *Lactobacillus viridescens*.² Their formulations led to the development of APT Agar.

Lactic acid bacteria, a group of acid-producing bacteria, include the genera *Streptococcus*, *Leuconostoc*, *Pediococcus*, and *Lactobacillus*.³ These organisms are widespread in nature, associated with bacterial spoilage of foods including dairy products, meat, and vegetables.³ APT Agar is used for cultivating heterofermentative lactic acid bacteria from food products.³

Principles of the Procedure

Enzymatic Digest of Casein and Yeast Extract are the carbon, nitrogen, and vitamin sources used for general growth requirements in APT Agar. Sodium Chloride maintains the osmotic balance of the medium. Potassium Phosphate is the buffering agent. Dextrose is the fermentable carbohydrate. Manganese Chloride, Magnesium Sulfate and Ferrous Sulfate provide ions used in replication by lactobacilli. Polysorbate 80 is a surfactant and a source of fatty acids required by lactobacilli. Sodium Carbonate is a neutralizing agent. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Yeast Extract.....	7.5 g
Sodium Chloride	5 g
Potassium Phosphate	5 g
Sodium Citrate	5 g
Dextrose.....	10 g
Polysorbate 80	0.2 g
Magnesium Sulfate	0.8 g
Manganese Chloride	0.14 g
Ferrous Sulfate	0.04 g
Sodium Carbonate	1.25 g
Agar	13.5 g

Final pH: 6.7 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 58 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 118 - 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is amber and trace to slightly hazy.

Expected Cultural Response: Cultural response on APT Agar at 35°C after 24 - 48 hours incubation.

Microorganism	Response
<i>Lactobacillus fermentum</i> ATCC® 9338	growth
<i>Leuconostoc mesenteroides</i> ATCC® 12291	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using APT Agar.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

APT Agar	Code No.	7302A	500 g
		7302B	2 kg
		7302C	10 kg

References

1. **Evans, J. B., and C. F. Niven, Jr.** 1951. Nutrition of the heterofermentative lactobacilli that cause greening of cured meat products. *J. Bact.* **62**:599-603.
2. **Deibel, R. H., J. B. Evans, and C. F. Niven, Jr.** 1957. Microbiological assay for thiamine using *Lactobacillus viridescens*. *J. Bact.* **74**:818-821.
3. **Vedamuthu, E. R., M. Raccach, B. A. Glatz, E. W. Seitz, and M. S. Reddy.** 1992. Acid-producing microorganisms, p. 225-238. In C. Vanderzant, and D. F. Splittstoesser (eds.). *Compendium of methods for the microbiological examination of foods*, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

AZIDE DEXTROSE BROTH (7315)

Intended Use

Azide Dextrose Broth is used in the detection of fecal streptococci in water, sewage, and milk.

Product Summary and Explanation

Roth originated the formula for Azide Dextrose Broth.¹ In a comparative study, Mallmann and Seligmann investigated the detection of streptococci in water and wastewater using Azide Dextrose Broth.² Their work supported the use of this medium in determining the presence of streptococci in water, wastewater, shellfish, and other materials. Azide Dextrose Broth has been used for primary isolation of streptococci from foods and other specimens of sanitary significance as an indication of fecal contamination.^{3,4}

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

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract are the carbon, nitrogen, and vitamin sources used for general growth requirements in Azide Dextrose Broth. 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 / Liter

Enzymatic Digest of Casein	7.5 g
Enzymatic Digest of Animal Tissue.....	7.5 g
Beef Extract	4.5 g
Dextrose.....	7.5 g
Sodium Chloride	7.5 g
Sodium Azide	0.2 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful by inhalation and if swallowed. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 34.7 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is yellow or gold to amber and clear.

Expected Cultural Response: Cultural response in Azide Dextrose Broth at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Streptococcus faecalis</i> ATCC® 19433	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure⁵

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°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.

Results

A positive test is indicated by turbidity (cloudiness) in the broth. A negative test remains clear. Azide Dextrose Broth tubes showing turbidity after 24 – 48 hours incubation must be subjected to the Confirmed Test Procedure. Consult appropriate references for details of the Confirmed Test Procedure⁵ and further identification of *Enterococcus*.^{5,6}

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

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}

Packaging

Azide Dextrose Broth	Code No.	7315A	500 g
		7315B	2 kg
		7315C	10 kg

References

1. **Roth**. 1948. Illinois State Health Department.
2. **Mallmann, W. L., and E. B. Seligmann**. 1950. A comparative study of media for the detection of streptococci in water and sewage. *Am. J. Public Health*. **40**:286.
3. **Larkin, E. P., W. Litsky, and J. E. Fuller**. 1955. Fecal streptococci in frozen foods. I. A bacteriological survey of some commercially frozen foods. *Appl. Microbiol.* **3**:98.
4. **Spittstoesser, D. F., R. Wright, and G. J. Hucker**. 1961. Studies on media for enumerating enterococci in frozen vegetables. *Appl. Microbiol.* **9**:303.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greensberg (eds.)**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BACILLUS CEREUS AGAR BASE (7442)

Intended Use

Bacillus Cereus Agar Base is used with polymyxin B and egg yolk suspension for the isolation and presumptive identification of *Bacillus cereus*.

Product Summary and Explanation

Holbrook and Anderson described a highly selective and diagnostic medium (PEMBA) for the isolation and determination of *Bacillus cereus* from food.¹ The medium is formulated to detect small numbers of *B. cereus* in the presence of large number of contaminants. This medium differentiates *B. cereus* from other bacteria based on resistance to polymyxin, lack of mannitol fermentation, and presence of lecithinase.^{2,3} *B. cereus* can be found on vegetables, processed foods, and in nature.⁴ *B. cereus* causes gastrointestinal illness if the organism is allowed to proliferate. Outbreaks of foodborne illness have been associated with boiled and cooked rice, cooked meats, and cooked vegetables.⁵

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein in Bacillus Cereus Agar Base. Sodium Chloride maintains the osmotic environment. Mannitol is the carbohydrate, and fermentation is detected by the pH indicator Bromthymol Blue. Magnesium Sulfate provides divalent cations and sulfate. The Phosphates are buffering agents in the medium. Sodium Pyruvate enhances growth and lecithinase production. Agar is the solidifying agent. Supplementing with Egg Yolk suspension provides lecithin, and Polymyxin B inhibits growth of most other bacteria. In the event of a high mold count, Cycloheximide (40 mg/L) can be added.

Formula / Liter

Enzymatic Digest of Casein	1 g
Mannitol	10 g
Sodium Chloride	2 g
Magnesium Sulfate	0.1 g
Disodium Phosphate.....	2.5 g
Monopotassium Phosphate	0.25 g
Bromthymol Blue.....	0.10 g
Sodium Pyruvate.....	10 g
Agar	15 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Sterile Egg Yolk Suspension, 50 mL
Polymyxin B (100,000 units), 2 mL
(filtered sterilized aqueous)

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 41 g of the medium in 950 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and aseptically add 50 mL of a sterile egg yolk suspension and 2 mL of a filtered sterilized aqueous solution of polymyxin B (100,000 units).

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and light green-beige.

Prepared Appearance: Prepared medium is yellow to yellow-green and opaque.

Expected Cultural Response: Cultural response on Bacillus Cereus Agar Base at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Bacillus cereus</i> ATCC® 13061	growth	blue colonies w/ halo
<i>Bacillus subtilis</i> ATCC® 9372	partial to complete inhibition	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the isolation and identification of *Bacillus cereus*.

Results

Bacteria that ferment mannitol produce acid products and form colonies that are yellow. Bacteria that produce lecithinase hydrolyze lecithin and a zone of white precipitate forms around the colonies. *B. cereus* is typically mannitol-negative (blue colonies) and lecithinase positive (zone of precipitate around colonies).

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Bacillus Cereus Agar Base	Code No.	7442A	500 g
		7442B	2 kg
		7442C	10 kg

References

1. **Holbrook and Anderson.** 1980. Can. J. Microbiol. **26**:753-759.
2. **Donovan, K. O.** 1958. A selective medium for *Bacillus cereus* in milk. J. Appl. Bacteriol. **21**:100-103.
3. **Coliner, A. R.** 1948. The action of *Bacillus cereus* and related species on the lecithin complex of egg yolk. J. Bacteriol. **55**:777-785.
4. **Jeffery, E. J. and S. M. Harmon.** 1995. *Bacillus cereus*, p. 14.01-14.08. In Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Harmon, S. M., J. M. Goepfert, and R. W. Bennett.** 1992. *Bacillus cereus*, p. 593-604. In C. Vanderzant, and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BAIRD PARKER AGAR (7112)

Intended Use

Baird Parker Agar is used for detection and enumeration of *Staphylococcus aureus* in foods.

Product Summary and Explanation

Baird Parker Agar was first described in 1962.¹ It is a selective medium for the isolation and presumptive identification of coagulase-positive staphylococci. This medium is used extensively for detecting *Staphylococcus aureus* in foods, dairy products, and other materials.²⁻⁶ Coagulase-positive staphylococci can grow and reproduce in cosmetic products. These products are tested for the presence of coagulase-positive staphylococci using standard microbiological methods.⁴

Principles of the Procedure

Enzymatic Digest of Casein and Beef Extract are the carbon and nitrogen sources in Baird Parker Agar. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Glycine and Sodium Pyruvate stimulate growth of staphylococci. The selectivity of the medium is due to Lithium Chloride and a 1% Potassium Tellurite Solution, suppressing growth of organisms other than staphylococci. The differentiation of coagulase-positive staphylococci is based on Potassium Tellurite and Egg Yolk Emulsion. Staphylococci that contain lecithinase break down the Egg Yolk and cause clear zones around the colonies. An opaque zone of precipitation may form due to lipase activity. Reduction of Potassium Tellurite is a characteristic of coagulase-positive staphylococci, and causes blackening of colonies. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Beef Extract	5 g
Yeast Extract.....	1 g
Lithium Chloride	5 g
Glycine	12 g
Sodium Pyruvate.....	10 g
Agar	17 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Enrichment

1% Potassium Tellurite Sol, 10 mL
Sterile Egg Yolk Emulsion, 50 mL

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful if swallowed, inhaled, or absorbed through skin. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 60 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. After cooling to 45 - 50°C, add 10 mL of a sterile 1% Potassium Tellurite Solution and 50 mL of sterile Egg Yolk Emulsion. Mix thoroughly before dispensing.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and light amber. The prepared enriched medium is canary yellow and opaque.

Expected Cultural Response: Cultural response on Baird Parker Agar with sterile 1% Potassium Tellurite Solution and sterile Egg Yolk Emulsion at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	growth	gray-black colonies, suppressed, no zone
<i>Escherichia coli</i> ATCC® 25922	inhibited	-----
<i>Proteus mirabilis</i> ATCC® 12453	growth	brown colonies
<i>Staphylococcus aureus</i> ATCC® 25923	growth	black colonies with a clear halo
<i>Staphylococcus epidermidis</i> ATCC® 12228	growth	black colonies, suppressed, no zone

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Prepare dilutions of test samples, if indicated by references.²⁻⁵
2. Transfer 1 mL of the sample to each of 3 Baird Parker Agar plates, distribute over the surface using a sterile, bent glass rod.
3. Allow inoculum to be absorbed by the medium before inverting the plates.
4. Incubate at 35 - 37°C for 45 - 48 hours.
5. Examine plates having 20 - 200 colonies, counting colonies typical of *Staphylococcus aureus*.

Results

Coagulase-positive staphylococci produce black, shiny, convex colonies with entire margins and clear zones, with or without an opaque zone. Coagulase-negative staphylococci produce poor or no growth. If growth occurs, colonies are black; clear or opaque zones are rare. The majority of other organisms are inhibited or grow poorly. If growth appears, colonies are light to brown-black, with no clear or opaque zones.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Baird Parker Agar	Code No.	7112A	500 g
		7112B	2 kg
		7112C	10 kg

References

1. **Baird-Parker, A. C.** 1962. An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bacteriol. **25**:12-19.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.
3. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
6. **United States Pharmacopeial Convention.** 1995. The United States Pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BCYE AGAR (7307) (LEGIONELLA AGAR)

Intended Use

BCYE Agar is used for the isolation of *Legionella* spp.

Product Summary and Explanation

In 1977, McDade et al. identified *Legionella pneumophila* as the causative agent of Legionnaires' disease, a multisystem disease manifested primarily by pneumonia.^{1,2} In 1978 a new medium, F-G Agar, resulted in improved growth of *L. pneumophila*, a very fastidious organism.³ Freely et al. modified F-C Agar by substituting yeast extract as a vitamin source and replacing starch with activated charcoal, producing Charcoal Yeast Extract (CYE) Agar.⁴ In 1980, Pasculle et al. reported that CYE Agar could be improved by the addition of ACES (N-2-acetamido-2-aminoethane sulfonic acid) buffer.⁵ One year later, Edelstein further increased the sensitivity of the medium by adding the potassium salt of alpha-ketoglutaric acid.⁶

Principles of the Procedure

Yeast Extract provides sources of nitrogen, carbon, and vitamins in BCYE Agar. Activated Charcoal decomposes hydrogen peroxide, a metabolic product toxic to *Legionella* spp., and may also collect carbon dioxide and modify surface tension. ACES Buffer is added to maintain the proper pH for optimal growth. α -Ketoglutarate stimulates organism growth. Agar is the solidifying agent. BCYE Agar is supplemented with L-Cysteine, an essential amino acid, and Ferric Pyrophosphate, an iron supplement. Both supplements are incorporated to satisfy specific nutritional requirements of *Legionella* spp. Selective agents can be added if necessary.

Formula / Liter

Yeast Extract..... 11.5 g
Charcoal, Activated..... 1.5 g
ACES Buffer..... 6 g
 α -Ketoglutarate 1 g
Agar 17 g

Final pH: 6.85 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements / 10 mL

L-Cysteine (4%), sterile
Ferric Pyrophosphate (2.5%), sterile

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 37 g of the medium in one liter of purified water.
2. Adjust pH to 6.8 – 6.9 with 1N KOH.
3. DO NOT heat prior to sterilization. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C.
4. Aseptically add 10 mL of a sterile solution of L-Cysteine (4%) and Ferric Pyrophosphate (2.5%).
5. Mix and determine pH. If necessary, aseptically adjust pH to 6.85 - 7.0 with a sterilized solution of 1N HCl or 1N KOH.
6. Add inhibitor solutions if required. Dispense with agitation.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and grey-black.

Prepared Appearance: Prepared medium is black and opaque.

Expected Cultural Response: Cultural response on BCYE Agar at 35°C after 72 - 96 hours incubation.

Microorganism	Response
<i>Legionella bozemanii</i> ATCC® 33217	growth
<i>Legionella dumoffii</i> ATCC® 33279	growth
<i>Legionella pneumophila</i> ATCC® 33152	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Culture the organism immediately upon arrival to the laboratory. Culture specimens from swabs by rolling the swab over a third of agar surface. Streak remainder of the plate to obtain isolated colonies. Incubate inoculated plates at $35 \pm 2^{\circ}\text{C}$ for a minimum of 3 days. Growth is usually visible within 3 - 4 days, but can take up to 2 weeks.

Results

Legionella pneumophila produces small to large, smooth, colorless to pale, blue-grey, slightly mucoid colonies that fluoresce yellow-green under longwave UV light. A gram stain, biochemical tests, and serological procedures should be performed for confirmation of *L. pneumophila*.

Storage

Store sealed bottle containing the dehydrated medium at $2 - 30^{\circ}\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Biochemical tests and serological procedures must be performed to confirm presence of *L. pneumophila*.

Packaging

BCYE Agar	Code No.	7307A	500 g
		7307B	2 kg
		7307C	10 kg

References

1. McDade, Shepard, Fraser, Tsai, Redus, Dowdle and the Laboratory Investigation Team. 1977. N. Engl. J. Med. **297**:1197.
2. Edelstein. 1985. In Lennette, Balows, Hausler and Shadomy (eds.). Manual of clinical microbiology, 4th ed. ASM. Washington, D.C.
3. Freely, Gorman, Weaver, Mackel and Smith. 1978. J. Clin. Microbiol. **8**:320.
4. Freely, Gibson, Gorman, Lansford, Rasheed, Mackel and Baine. 1979. J. Clin. Microbiol. **10**:437.
5. Pasculle, Freely, Gibson, Cordes, Myerowitz, Patton, Gorman, Carmack, Ezzell and Dowling. 1980. J. Infect. Dis. **141**:727.
6. Edelstein. 1981. J. Clin. Microbiol. **14**:298.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BEEF EXTRACT POWDER (7228)

Intended Use

Beef Extract Powder is a dehydrated extract of bovine tissue for use in preparing microbiological culture media.

Product Summary and Explanation

Beef Extract Powder is prepared and standardized for use in microbiological culture media, where it is generally used to replace infusion of meat. Culture media containing Beef Extract Powder are recommended for use in bacteriological examination of water, milk, and other materials, where uniform composition of media is important. Beef Extract Powder is relied upon for biochemical studies, particularly fermentation reactions because of its independence from fermentable substances. Several media containing Beef Extract Powder are recommended in standard methods for multiple application.¹⁻³

Principles of the Procedure

Beef Extract Powder provides nitrogen, amino acids, vitamins, and carbon. Beef Extract Powder is usually employed in concentrations of 0.3% in culture media. Concentrations may vary slightly according to the requirements of individual formulas, but do not often exceed 0.5%.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and dark beige to tan.

Prepared Appearance (2% wt/vol): Prepared medium is clear, amber with no or a light precipitate.

pH (2% Solution at 25°C): 6.5 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35° C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to excellent growth

Test Procedure

Refer to appropriate references for specific procedures using Beef Extract Powder.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Beef Extract Powder at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Beef Extract Powder should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to Beef Extract Powder in its intact container when stored as directed.

Packaging

Beef Extract Powder	Code No.	7228A	500 g
		7228B	2 kg
		7228C	10 kg

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BETA-SSA AGAR (7336)

Intended Use

Beta-SSA Agar is used with blood for the selective isolation of group A streptococci.

Product Summary and Explanation

Group A streptococcal infections are the most common cause of bacterial pharyngitis in children 5 to 10 years old.¹ Beta-SSA Agar is a highly selective agar developed for the isolation of group A beta-hemolytic streptococci. The selective agents in the medium inhibit gram-negative bacteria and most Gram-positive bacteria, although some strains of group B beta-hemolytic streptococci may grow. Beta-SSA Agar is supplemented with 5% sheep blood to detect hemolytic patterns of streptococci.

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal. Sodium Chloride maintains the osmotic balance of the medium. Selective Agents inhibit gram-negative bacteria and most gram-positive bacteria. Agar is the solidifying agent.

In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β -hemolytic streptococci.² Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and type of basal medium used.¹

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Selective Agents	0.0462 g
Agar	15 g

Final pH: 7.3 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium without blood is beige and trace to slightly hazy. With 5% sheep blood the medium is red and opaque.

Expected Cultural Response: Cultural response on Beta-SSA Agar supplemented with 5% sheep blood at 35°C after 18 - 24 hours incubation.

<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---
<i>Streptococcus pneumoniae</i> ATCC® 6305	inhibited	---
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis (clear zone w/ Bacitracin disk)

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.¹
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results³

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Atmosphere of incubation is known to influence hemolytic reactions of beta-hemolytic streptococci.¹ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Packaging

Beta-SSA Agar	Code No.	7336A	500 g
		7336B	2 kg
		7336C	10 kg

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
2. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
3. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BIGGY AGAR (7191)

Intended Use

BIGGY Agar is used for the isolation and differentiation of *Candida* spp.

Product Summary and Explanation

BIGGY Agar is a modification of the formula described by Nickerson.^{1,2} BIGGY Agar, an abbreviation for Bismuth Glucose Glycine Yeast Agar, is also referred to as Nickerson Agar and Nickerson Candida Selective Agar.

BIGGY Agar was developed while studying sulfite reduction by *Candida* spp. Nickerson found that *Candida albicans* can be differentiated from other *Candida* spp. on this medium based on colony morphology.

Candidiasis, the most frequently encountered opportunistic fungal infection is usually caused by *Candida albicans*.³ *Candida tropicalis* and *Candida (Torulopsis) glabrata* infections occur less frequently.³ *Candida* spp. are present in clinical specimens as a result of environmental contamination, colonization, or an actual disease process.⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon, source is provided by Yeast Extract in BIGGY Agar. Glycine is used to stimulate growth. Dextrose is the carbohydrate source. *Candida* spp. reduce Bismuth Ammonium Citrate, and colonies become brown to black in color. Bismuth Ammonium Citrate and Sodium Sulfite are selective agents against bacteria, often present as normal flora. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	1 g
Glycine	10 g
Dextrose.....	10 g
Bismuth Ammonium Citrate	5 g
Sodium Sulfite.....	3 g
Agar	16 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful by inhalation, ingestion, and skin contact.

Directions

1. Suspend 45 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to moderately hazy, and grey-white in color.

Expected Cultural Response: Cultural response at 30°C after 18 - 72 hours incubation.

Microorganism	Response	Reactions
<i>Candida albicans</i> ATCC® 10231	growth	brown-black, no diffusion w/ mycelial fringe
<i>Candida tropicalis</i> ATCC® 750	growth	dark brown w/ black center and black diffusion around colonies
<i>Escherichia coli</i> ATCC® 25922	inhibited	--
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and identification of yeast spp.^{3,4}

Results

Colony morphology, according to Nickerson,² after 48 hours of incubation on BIGGY Agar:

<i>C. albicans</i>	Intensely brown-black colonies with slight mycelial fringe, medium sized, no diffusion.
<i>C. tropicalis</i>	Discrete dark brown colonies with black centers, and sheen, medium sized, diffuse blackening of the surrounding medium after 72 hours of incubation.
<i>C. pseudotropicalis</i>	Large, dark red-brown colonies, flat, with slight mycelial fringe.
<i>C. krusei</i>	Large, flat, wrinkled colonies with silver-black top, brown edge, and yellow halo.
<i>C. parakrusei</i>	Medium size, flat, wrinkled colonies with red-brown color, and yellow mycelial fringe.
<i>C. stellatoidea</i>	Medium size, flat, dark brown colonies, very light mycelial fringe.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Pigmented bacterial and yeast-like fungi are usually inhibited on BIGGY Agar. They can be differentiated by microscopic examination. Dermatophytes and molds seldom appear, and are easily recognized by development of aerial mycelia.⁵
3. Further growth characteristic and biochemical tests are needed to differentiate yeasts, particularly identification of *Candida* spp.⁵
4. BIGGY Agar should be inoculated when the medium is freshly prepared.^{1,2}
5. Do not prepare slants of BIGGY Agar, the reactions are unsatisfactory.^{1,2}

Packaging

BIGGY Agar	Code No.	7191A	500 g
		7191B	2 kg
		7191C	10 kg

References

1. **Nickerson, W. J.** 1947. Biology of pathogenic fungi. The Chronica Botanica Co., Waltham, MA.
2. **Nickerson, W. J.** 1953. Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulfite by species of *Candida*. J. Infect. Dis. **93**:43.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
4. **Warren, N. G., and K. C. Hazen.** 1995. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 723-737. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 65-68. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BILE ESCULIN AGAR (7249)

Intended Use

Bile Esculin Agar is used for the selective isolation and differentiation of group D streptococci.

Product Summary and Explanation

Bile Esculin Agar is based on the formulation described by Swan and further evaluated by Facklam and Moody.^{1,2} Rochaix first noted the value of esculin hydrolysis in the identification of enterococci.³ Meyer and Schonfeld added bile to the esculin medium and demonstrated 61 of 62 enterococci strains were able to grow and hydrolyze esculin, while other streptococci could not.⁴

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described as group D streptococci, in the genus *Enterococcus*.⁵ The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci. Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping,¹ and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction. Facklam and Moody found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.²

Bile Esculin Agar is in standard procedures for the microbiological examination of food products.⁶⁻⁸

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxbile is used to inhibit gram-positive bacteria other than enterococci. Beef Extract and Enzymatic Digest of Gelatin are the carbon and nitrogen sources used for general growth requirements in Bile Esculin Agar. Agar is the solidifying agent.

Formula / Liter

Beef Extract	11 g
Enzymatic Digest of Gelatin	34.5 g
Esculin	1 g
Oxbile	2 g
Ferric Ammonium Citrate	0.5 g
Agar	15 g

Final pH: 6.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 64 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to dark beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, opalescent, and grey-yellow.

Expected Cultural Response: Cultural response on Bile Esculin Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction Esculin Hydrolysis
<i>Enterococcus faecalis</i> ATCC® 19433	growth	+ (black colonies)
<i>Enterococcus faecalis</i> ATCC® 29212	growth	+ (black colonies)
<i>Enterococcus faecalis</i> ATCC® 33186	growth	+ (black colonies)
<i>Escherichia coli</i> ATCC® 25922	growth	-
<i>Streptococcus pyogenes</i> ATCC® 19615	inhibited	-

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for instructions on specific material being tested for group D streptococci.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Bile Esculin Agar	Code No.	7249A	500 g
		7249B	2 kg
		7249C	10 kg

References

1. **Swan, A.** 1954. The use of bile-esculin medium and of Moxed's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. **7**:160.
2. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. **20**:245.
3. **Rochaix, A.** 1924. Milieux a leculine pour le diagnostid differentiel des bacteries du grojps strepto-entero-pneumocoque. Comt. Rend. Soc. Biol. **90**:771-772.
4. **Meyer, K., and H. Schönfeld.** 1926. Über die Unterscheidung des Enterococcus vom Streptococcus viridans und die Beziehung beider zum Strptoccus lactis. Zentralb. Bakteriell Parasitenkd. Infektionskr. Hyg. Abt. I orig. **99**:402-416.
5. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. **10**:1-19.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, MD.
8. **Marshall, R. T. (ed.).** 1992. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BILE ESCULIN AZIDE AGAR (7133)

Intended Use

Bile Esculin Azide Agar is used for the selective isolation and differentiation of group D streptococci.

Product Summary and Explanation

Bile Esculin Azide Agar is a modification of the medium reported by Isenberg and Isenberg, Goldberg, and Sampson.^{1,2} This formula modifies Bile Esculin Agar by adding sodium azide and reducing the concentration of bile. The revised medium is more selective, but still provides rapid growth and efficient recovery of group D streptococci.

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described group D streptococci, in the genus *Enterococcus*.³ The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci. Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping, and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.⁴ Facklam and Moody found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.⁵

Sabbaj, Sutter, and Finegold evaluated selective media for selectivity, sensitivity, detection, and enumeration of presumptive group D streptococci from human feces.⁶ Bile Esculin Azide Agar selected for *S. bovis*, displayed earlier distinctive reactions, and eliminated the requirement for special incubation temperatures.

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxbile is used to inhibit gram-positive bacteria other enterococci, while Sodium Azide inhibits gram-negative bacteria. Enzymatic Digest of Casein and Yeast Enriched Meat Peptone are the carbon, nitrogen, and vitamin sources used for general growth requirements in Bile Esculin Agar. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate acts as a preservative. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	25 g
Yeast Enriched Meat Peptone	9.5 g
Oxbile.....	1 g
Sodium Chloride	5 g
Sodium Citrate	1 g
Ferric Ammonium Citrate.....	0.5 g
Esculin	1 g
Sodium Azide	0.25 g
Agar	14 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL BY INHALATION AND IF SWALLOWED. Avoid contact with skin and eyes.

Directions

1. Suspend 56 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to dark beige.

Prepared Appearance: Prepared medium is grey to green-yellow, opalescent, and trace to slightly hazy.

Expected Cultural Response: Cultural response on Bile Esculin Azide Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction Esculin Hydrolysis
<i>Enterococcus faecalis</i> ATCC® 29212	growth	+ (blackening around colonies)
<i>Escherichia coli</i> ATCC® 25922	inhibited	-
<i>Streptococcus pyogenes</i> ATCC® 19615	partial to complete inhibition	- (colorless colonies)

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for instructions on specific material being tested for group D streptococci.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Bile Esculin Azide Agar	Code No.	7133A	500 g
		7133B	2 kg
		7133C	10 kg

References

1. **Isenberg, H. D.** 1970. Clin. Lab. Forum.
2. **Isenberg, H. D., D. Goldberg, and J. Sampson.** 1970. Laboratory studies with a selective enterococcus medium. Appl. Microbiol. **20**:433.
3. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. **10**:1-19.
4. **Swan, A.** 1954. The use of bile-esculin medium and of Moxed's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. **7**:160.
5. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. **20**:245.
6. **Sabbaj, J., V. L. Sutter, and S. M. Finegold.** 1971. Comparison of selective media for isolation of presumptive group D streptococci from human feces. Appl. Microbiol. **22**:1008.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BILE SALTS MIXTURE #3 (7230)

Intended Use

Bile Salts Mixture #3 is a mixture of bile salts for use in preparing microbiological culture media.

Product Summary and Explanation

Bile Salts Mixture #3 is a mixture of sodium cholate and sodium deoxycholate. It's primary use is as a selective agent for the isolation of gram-negative microorganisms, inhibiting gram-positive cocci. Bile Salts Mixture #3 is prepared especially for use in MacConkey Agar and Violet Red Bile Agar. Bile Salts Mixture #3 is soluble in distilled or deionized water and neutral to slightly alkaline in pH. It is used in preparing culture media without pre-filtration or adjustment of reaction. Several media containing Bile Salts Mixture #3 are recommended in standard methods for multiple applications.¹⁻³

Principles of the Procedure

Bile is derived from the liver. Bile Salts Mixture #3 contains bile extract standardized to provide inhibitory properties for selective media. Bile Salts Mixture #3 inhibits the growth of gram-positive organisms and spore-forming bacilli without affecting the development of gram-negative enteric bacilli.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and white in color.

Prepared Appearance (2.0 % wt/vol): Prepared medium is clear, colorless to light amber without a precipitate.

pH (2% Solution at 25°C): 7.8 - 8.6

Expected Cultural Response: Cultural response in MacConkey Agar after incubation at 35°C for 18 - 24 hours.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth, pink to red colonies surrounded by a zone of precipitated bile
<i>Staphylococcus aureus</i> ATCC 25923	complete inhibition

Test Procedure

Refer to appropriate references for specific procedures using Bile Salts Mixture #3.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Bile Salts Mixture #3 at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Bile Salts Mixture #3 should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Bile Salts Mixture #3 in its intact container when stored as directed.

Packaging**Bile Salts Mixture #3****Code No.****7230A****500 g****7230B****2 kg****7230C****10 kg****References**

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BISMUTH SULFITE AGAR (7113)

Intended Use

Bismuth Sulfite Agar is used for the selective isolation of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and/or neurological damage.¹ Salmonellosis can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella*. U. S. federal guidelines require various poultry products to be routinely monitored before distribution for human consumption.

Bismuth Sulfite Agar is a modification of Wilson and Blair formula.²⁻⁴ The typhoid organism grows luxuriantly on the medium, forming characteristic black colonies. Gram-positive bacteria and coliforms are inhibited on Bismuth Sulfite Agar. The inhibitory action of Bismuth Sulfite Agar permits the use of a large inoculum, increasing the possibility of recovering pathogens that may be present in small numbers. Bismuth Sulfite Agar is generally accepted for routine detection of most *Salmonella* spp. Bismuth Sulfite Agar is used for the isolation of *S. typhi* and other *Salmonella* spp. from food, feces, urine, sewage, and other infectious materials. Bismuth Sulfite Agar is a standard methods medium for industrial applications and the clinical environment.⁵⁻⁹

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide sources of nitrogen, carbon, and vitamins required for organism growth. Dextrose is the carbohydrate present in Bismuth Sulfite Agar. Disodium Phosphate is the buffering agent. Bismuth Sulfite Indicator and Brilliant Green are complementary, inhibiting gram-positive bacteria and coliforms, allowing *Salmonella* spp. to grow. Ferrous Sulfate is used for H₂S production. When H₂S is present, the iron in the formula is precipitated, and positive cultures produce the characteristic brown to black color with metallic sheen. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Beef Extract	5 g
Dextrose.....	5 g
Disodium Phosphate.....	4 g
Ferrous Sulfate	0.3 g
Bismuth Sulfite Indicator	8 g
Brilliant Green	0.025 g
Agar	20 g

Final pH: 7.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful by inhalation, ingestion, and skin contact.

Directions

1. Suspend 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute.
3. Mix thoroughly to obtain a uniform suspension prior to dispensing.
4. Prepared plates may be used the same day as prepared.
5. For increased selectivity, current references suggest that prepared BSA plates be stored overnight in the dark at room temperature.⁸

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green-beige.

Prepared Appearance: Prepared medium at 45 - 50°C is green-yellow and opaque.

Expected Cultural Response: Cultural response on Bismuth Sulfite Agar at 35°C after 40 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	---
<i>Escherichia coli</i> ATCC® 25922	partially inhibited	brown-green colonies
<i>Salmonella typhimurium</i> ATCC® 19430	growth	black colonies with metallic sheen
<i>Shigella flexneri</i> ATCC® 12022	growth	brown colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For isolation of *Salmonella typhi* and other *Salmonella* spp. consult appropriate references.

Results

Typical *S. typhi* surface colonies are black, surrounded by black or brown-black zone. This zone may be several times the size of the colony. Other strains of *Salmonella* produce black to green colonies with little or no darkening of surrounding medium. *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.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Streak for well isolated colonies. In heavy growth areas, *S. typhi* appears light green and may be misinterpreted as negative for *S. typhi*.¹⁰
2. *S. typhi* and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. However, *S. arizonae* is usually inhibited.¹⁰ 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.¹⁰
3. Do not autoclave medium. Heating Bismuth Sulfite Agar for a period longer than necessary may destroy selectivity properties.

Packaging

Bismuth Sulfite Agar	Code No.	7113A	500 g
		7113B	2 kg
		7113C	10 kg

References

1. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. **Wilson, W. J., and E. M. Blair.** 1926. A combination of bismuth and sodium sulphite affording an enrichment and selective medium for the typhoid-paratyphoid groups of bacteria. J. Pathol. Bacteriol. **29**:310.
3. **Wilson, W. J., and E. M. Blair.** 1927. Use of a glucose bismuth sulphite iron medium for the isolation of *B. typhosus* and *B. proteus*. J. Hyg. **26**:374-391.
4. **Wilson, W. J., and E. M. Blair.** 1931. Further experience of the bismuth sulphite media in the isolation of *B. typhosus* and *B. proteus*. J. Hyg. **31**:138-161.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. **Vanderzant, C., and D.F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
8. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. In FDA Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
9. **Cunniff, P. (ed.).** Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
10. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BLOOD AGAR BASE NO. 2 (7266)

Intended Use

Blood Agar Base NO. 2 is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Blood agar bases are 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, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.¹ Blood Agar Base NO. 2 is a nutritionally rich medium for maximum recovery of fastidious microorganisms. Blood Agar Base media are specified in standard method procedures for food testing.²⁻⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Animal Tissue, Enzymatic Digest of Casein, Liver Digest, and Yeast Extract in Blood Agar Base NO. 2. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	7.5 g
Enzymatic Digest of Animal Tissue.....	7.5 g
Liver Digest	2.5 g
Yeast Extract.....	5 g
Sodium Chloride	5 g
Agar	12 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 39.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light amber, and trace to slightly hazy. With 5% sheep blood, medium is cherry red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Based No. 2 with 5% sheep blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	-
<i>Neisseria meningitidis</i> ATCC® 13090	growth	-
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for oxygen-stable and oxygen-labile streptolysins.⁵
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁶

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (α') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance or has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁵
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.⁵ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%), in accordance with established laboratory procedures.

Packaging

Blood Agar Base NO. 2	Code No.	7266A	500 g
		7266B	2 kg
		7266C	10 kg

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
4. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
6. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BLOOD AGAR BASE w/ LOW pH (7354)

Intended Use

Blood Agar Base w/ LOW pH is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Blood agar bases are 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, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.¹ Lowering the pH in Blood Agar Base w/ LOW pH is used to enhance hemolytic reactions of streptococci.² Blood Agar Base media are specified in standard method procedures for food testing.³⁻⁵

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Heart Infusion Solids and Enzymatic Digest of Animal Tissue in Blood Agar Base w/ LOW pH. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Heart Infusion Solids 10 g
Enzymatic Digest of Animal Tissue 10 g
Sodium Chloride 5 g
Agar 15 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light amber, clear to slightly hazy, without significant precipitate. With 5% sheep blood, medium is cherry red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Base w/ LOW pH with 5% sheep blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis-
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.⁶
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁷

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (α') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁶
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.⁶ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%).

Packaging

Blood Agar Base w/ LOW pH	Code No.	7354A	500 g
		7354B	2 kg
		7354C	10 kg

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Norton, J. F.** 1932. Bacteriology of pus. J. Lab. Clin. Med. **17**:558-564.
3. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
5. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
7. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BLOOD AGAR BASE, IMPROVED (7268)

Intended Use

Blood Agar Base, Improved is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Blood agar bases are 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, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.¹ In Blood Agar Base, Improved, the formula was modified slightly to enhance organism growth and neutralize any toxic metabolites. Blood Agar Base media are specified in standard method procedures for food testing.²⁻⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue in Blood Agar Base, Improved. Yeast Extract is a vitamin source. Corn Starch is added to ensure any toxic metabolites produced are absorbed, and enhance organism growth.⁵ Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Animal Tissue.....	4 g
Yeast Extract.....	2 g
Corn Starch.....	1 g
Sodium Chloride	5 g
Agar	14 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light amber and hazy. With 5% sheep blood, medium is cherry red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Base, Improved with 5% sheep blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	---
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.⁶
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁷

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (α') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁶
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.⁶ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%).

Packaging

Blood Agar Base, Improved	Code No.	7268A	500 g
		7268B	2 kg
		7268C	10 kg

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
4. **Greenberg, A. E., L. S. Clesceri, and A.D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 141-143. Williams & Wilkins, Baltimore, MD.
6. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
7. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BLOOD AGAR BASE, pH 7.4 (7114)

Intended Use

Blood Agar Base, pH 7.4 is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Blood agar bases are 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, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.¹ Blood Agar Base media are specified in standard method procedures for food testing.²⁻⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Heart Muscle Infusion and Enzymatic Digest of Animal Tissue in Blood Agar Base, pH 7.4. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Heart Muscle Infusion	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Sodium Chloride	5 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light amber, trace to slightly hazy. With 5% sheep blood, medium is cherry red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Base, pH 7.4 with 5% sheep blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	---
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.⁵
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁶

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (α') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁵
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.⁵ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%).

Packaging

Blood Agar Base, pH 7.4	Code No.	7114A	500 g
		7114B	2 kg
		7114C	10 kg

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
4. **Greenberg, A. E., L. S. Clesceri, and A.D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
6. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRAIN-HEART INFUSION AGAR (7115)

Intended Use

Brain-Heart Infusion Agar is used for the cultivation of a wide variety of fastidious organisms.

Product Summary and Explanation

Rosenow prepared a rich medium for culturing streptococci by combining dextrose broth and brain tissue.¹ Hayden modified the original formula while working with dental pathogens.² The current formula is a modification of Rosenow and Hayden, using dehydrated infusions of pork brain and pork heart tissue.

Brain-Heart Infusion Agar can be supplemented with antibiotics, varying amounts of sodium chloride, yeast extract and serum to provide a rich medium for bacteria, yeast, and pathogenic fungi.³ Brain-Heart Infusion Agar (BHI Agar), is specified in many references for food and water testing.⁴⁻⁶ Standard Methods for the Examination of Water and Wastewater recommends Brain-Heart Infusion media in tests for the verification of fecal streptococci.⁷

Principles of the Procedure

The nitrogen, vitamin, and carbon source is provided by Brain Heart Infusion, Enzymatic Digest of Animal Tissue, and Enzymatic Digest of Casein in BHI Agar. Dextrose is the carbohydrate source, and Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent.

Formula / Liter

Brain Heart Infusion (Solids).....	8 g
Enzymatic Digest of Animal Tissue.....	5 g
Enzymatic Digest of Casein	16 g
Dextrose.....	2 g
Sodium Chloride	5 g
Disodium Phosphate.....	2.5 g
Agar	13.5 g

Final pH 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, and light to medium amber.

Expected Cultural Response: Cultural response on Brain-Heart Infusion Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Candida albicans</i> ATCC® 10231	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Brain-Heart Infusion Agar.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brain-Heart Infusion Agar	Code No.	7115A	500 g
		7115B	2 kg
		7115C	10 kg

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Research **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 147-153, CRC Press, Boca Raton, FL.
4. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
5. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.
7. **Greenberg, A. E., L. S. Clesceri, and A.D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRAIN-HEART INFUSION BROTH (7116)

Intended Use

Brain-Heart Infusion Broth is used for the cultivation of a wide variety of fastidious organisms.

Product Summary and Explanation

Rosenow¹ prepared a rich medium for culturing streptococci by combining dextrose broth and brain tissue. Hayden² modified the original formula while working with dental pathogens. The current formula is a modification of Rosenow¹ and Hayden², using dehydrated infusions of porcine brain and heart tissue.

Brain-Heart Infusion Broth can be supplemented with antibiotics, varying amounts of sodium chloride, yeast extract, and serum to provide a rich medium for bacteria, yeasts and pathogenic fungi.³ The addition of 0.1% agar can be used to lower oxygen tension, providing an atmosphere to support the growth of aerobic, microaerophilic, and obligate anaerobic microorganisms.

Brain-Heart Infusion Broth, abbreviated as BHI, is specified in many references for food and water testing.⁴⁻⁷ NCCLS, National Committee for Clinical Laboratory Standards, cites Brain-Heart Infusion Broth for preparing the inoculum used in antimicrobial susceptibility tests.⁸

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Brain-Heart Infusion and Enzymatic Digest of Gelatin in BHI Broth. Dextrose is the carbohydrate source, and Sodium Chloride maintains the osmotic environment. Disodium Phosphate is the buffering agent in this medium.

Formula / Liter

Brain Heart Infusion 17.5 g
Enzymatic Digest of Gelatin 10 g
Dextrose..... 2 g
Sodium Chloride 5 g
Disodium Phosphate..... 2.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 37 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared broth is clear, with and without a minor precipitate, and light to medium amber in color.

Expected Cultural Response: Cultural response at 35°C for 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Brain-Heart Infusion Broth.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brain-Heart Infusion Broth	Code No.	7116A	500 g
		7116B	2 kg
		7116C	10 kg

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Research **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 147-153, CRC Press, Boca Raton, FL.
4. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
5. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.
7. **Greenberg, A. E., L. S. Clesceri, and A.D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
8. **National Committee for Clinical Laboratory Standards.** 1994. M11-A3, Vol. 13, No. 26, Methods for antimicrobial susceptibility testing of anaerobic bacteria. National Committee for Clinical Laboratory Standards, Villanova, PA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRAIN-HEART INFUSION SOLIDS (7262)

Intended Use

Brain-Heart Infusion Solids is dehydrated infusion of porcine brains and hearts for use in preparing microbiological culture media.

Product Summary and Explanation

Rosenow devised an excellent medium for culturing streptococci by supplementing Dextrose Broth with bovine brain tissue.¹ Hayden, revising Rosenow's procedure by adding crushed marble to the medium, reported favorable growth of organisms from dental pathogens. Brain-Heart Infusion Solids was developed as an alternative to bovine based Brain Heart Infusion containing media, replacing calf brain and beef heart with porcine brains and hearts. Brain-Heart Infusion Solids was developed for pharmaceutical and vaccine production, and can replace traditional bovine based BHI media in most applications.

The nutritionally rich formula of Brain-Heart Infusion Solids 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

Brain-Heart Infusion Solids provides nitrogen, amino acids, and vitamins in microbiological culture media. Brain-Heart Infusion Solids is processed from large volumes of raw material, retaining all the nutritive and growth stimulating properties of fresh tissue.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogenous, free-flowing beige.

Prepared Appearance (2% wt/vol): Prepared medium is clear, amber with no or a light precipitate.

pH (2% Solution at 25°C): 6.5 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to good growth

Test Procedure

Refer to appropriate references for specific procedures using Brain-Heart Infusion Solids.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Brain-Heart Infusion Solids at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Brain-Heart Infusion Solids should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Brain-Heart Infusion Solids in its intact container when stored as directed.

Packaging

Brain-Heart Infusion Solids	Code No.	7262A	500 g
		7262B	2 kg
		7262C	10 kg

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Res. **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Cunniff, P. (ed.).** 1995. Official methods of analysis, AOAC International, 16th ed. AOAC International, Arlington, VA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRAIN-HEART INFUSION w/o DEXTROSE (7193)

Intended Use

Brain-Heart Infusion w/o Dextrose is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Rosenow¹ prepared a rich medium for culturing streptococci by combining dextrose broth and brain tissue. Hayden² modified the original formula while working with dental pathogens. The current formula is a modification of Rosenow¹ and Hayden,² using dehydrated infusions of pork brain and heart tissue.

Brain-Heart Infusion w/o Dextrose is a basal medium used with added carbohydrates for fermentation studies, or supplemented with blood. Brain-Heart Infusion media is specified in standard methods.³⁻⁵

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Brain-Heart Infusion and Enzymatic Digest of Gelatin. Sodium Chloride maintains the osmotic environment. Dipotassium Phosphate is the buffering agent in this medium.

Formula / Liter

Brain-Heart Infusion (dehydrated).....	17.5 g
Enzymatic Digest of Gelatin	10 g
Sodium Chloride	5 g
Dipotassium Phosphate	2.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 35 g of the medium in one liter of purified water until evenly dissolved.
2. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear with and without a minor precipitate and light to medium amber.

Expected Cultural Response: Cultural response in Brain-Heart Infusion w/o Dextrose at 35°C after 18 - 96 hours incubation.

Microorganism	Response
<i>Haemophilus parasuis</i> ATCC® 19417	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Brain-Heart Infusion w/o Dextrose or carbohydrate fermentation studies.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brain-Heart Infusion w/o Dextrose	Code No.	7193A	500 g
		7193B	2 kg
		7193C	10 kg

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Research **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
4. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRILLIANT GREEN AGAR (7117)

Intended Use

Brilliant Green Agar is used for the selective isolation of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *Salmonella typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and/or neurological damage.¹ Infection can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella*. U. S. federal guidelines require various poultry products routinely monitored before distribution for human consumption.

Kristensen, Lester, and Jurgens first described the use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella*.² The report described the medium as useful for the differentiation of "paratyphoid B" from other intestinal gram-negative bacilli.² Kaufmann modified their formula and used Brilliant Green Agar in addition to Tetrathionate Broth for the isolation of *Salmonella* from stool specimens.³

Brilliant Green Agar is recommended for use in testing clinical specimens.^{1,4} The outstanding selectivity of this medium permits use of moderately heavy inocula, which should be evenly distributed over the surface. Brilliant Green Agar is used in the microbial limits test.⁵ Brilliant Green Agar supplemented with novobiocin is used in food testing.⁶

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide sources of nitrogen, amino acids, and carbon. Yeast Extract supplies vitamins required for organism growth. Sodium Chloride maintains the osmotic balance of the medium. Lactose and Sucrose are the carbohydrates in the medium. Brilliant Green inhibits gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Phenol Red is the pH indicator and turns the medium yellow with the formation of acid when lactose and/or sucrose is fermented. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Lactose	10 g
Sucrose.....	10 g
Brilliant Green	0.0125 g
Phenol Red	0.08 g
Agar	20 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 58 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a green tint.

Prepared Appearance: Prepared medium is brown-green, slightly opalescent, and may be trace to slightly hazy.

Expected Cultural Response: Cultural response on Brilliant Green Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	partial inhibition	green colonies
<i>Salmonella choleraesuis</i> ATCC® 13076	growth	pink colonies
<i>Salmonella typhi</i> ATCC® 19430	partial inhibition	pink colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	pink colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For isolation of *Salmonella* from clinical specimens, inoculate fecal specimens and rectal swabs on the first quadrant of Brilliant Green Agar and streak for isolation. Incubate plates at 35°C. Examine plates after 18 – 24 hours for colonies with characteristic morphologies associated with *Salmonella* spp. Refer to appropriate references or standard methods for other applications.

Results

Typical *Salmonella* spp. colonies are opaque and pink. The few lactose and/or sucrose fermenting Organisms that grow are readily differentiated due to formation of green colonies. Brilliant Green Agar is not suitable for the isolation of *S. typhi* or *Shigella* spp., although some strains of *S. typhi* may grow.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Colonies of *Salmonella* spp. can be red, pink, or white depending on length of incubation and strain.⁷
2. Medium is normally orange-brown, however after incubation it can turn bright red and return to normal color at room temperature.⁷
3. Taylor showed that slow lactose fermenters, *Proteus*, *Citrobacter*, and *Pseudomonas* may grow on Brilliant Green Agar as red colonies.⁸
4. Other primary plating medium such as MacConkey Agar should be used when testing for intestinal pathogens. Fluid enrichments, such as Selenite Broth, should be used with Brilliant Green.

Packaging

Brilliant Green Agar	Code No.	7117A	500 g
		7117B	2 kg
		7117C	10 kg

References

1. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. **Kristensen, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren Fur Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville. MD.
6. **Federal Register.** 1993. Chicken disease caused by *Salmonella enteritidis*; proposed rule. Fed. Regist. **58**:41048-41061.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.
8. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars: New media for isolation of enteric pathogens. Am J. Clin. Pathol. **44**:471.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRILLIANT GREEN AGAR W/ SULFADIAZINE (7310)

Intended Use

Brilliant Green Agar w/ Sulfadiazine is used for the selective enrichment of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem. Infection with non-typhi *Salmonella* spp. often causes mild, self-limiting illness.¹ 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.

Brilliant Green Agar was first described by Kristensen et al.² and later modified by Kauffman.³ The outstanding selectivity of this medium permits the use of moderately heavy inocula, evenly distributed over the surface. The addition of sulfonamides into Brilliant Green Agar further inhibits *Escherichia coli* and *Proteus* spp. Brilliant Green Agar, abbreviated as BGA, with Sulfadiazine is recommended for the isolation of *Salmonella* from foods, especially eggs.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are the carbon and nitrogen sources used for general growth requirements in BGA w/ Sulfadiazine. Yeast Extract supplies B-complex vitamins, and Lactose and Sucrose are the carbohydrates in this medium. In the presence of Phenol Red, a pH indicator, nonlactose and/or nonsucrose-fermenting *Salmonella* will produce red colonies. Sulfadiazine and Brilliant Green are the selective agents, inhibiting gram-positive organisms and many gram-negative bacteria, except *Salmonella*. Sodium Chloride maintains the osmotic balance. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Lactose	10 g
Sucrose.....	10 g
Brilliant Green	0.0125 g
Phenol Red	0.08 g
Sulfadiazine.....	0.08 g
Agar	20 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 58 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a green tint.

Prepared Appearance: Prepared medium is brown-green and slightly opalescent.

Expected Cultural Response: Cultural response on Brilliant Green Agar w/ Sulfadiazine at 35°C for 18 - 24 hours incubation.

Microorganism	Response	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	----
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	yellow to green colonies
<i>Salmonella choleraeuis</i> ATCC® 13076	good growth	red colonies
<i>Salmonella typhi</i> ATCC® 19430	poor growth	red colonies
<i>Salmonella typhimurium</i> ATCC® 14028	good growth	red colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	----

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for instructions on specific material being tested for *Salmonella*.^{1,4-7}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brilliant Green Agar w/ Sulfadiazine	Code No.	7310A	500 g
		7310B	2 kg
		7310C	10 kg

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
2. **Kristense, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, bromthymol blue, bromcresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **U. S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRILLIANT GREEN BILE BROTH 2% (7119)

Intended Use

Brilliant Green Bile Broth 2% is used for the detection of coliform bacteria in water, food, and dairy products.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative 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 this group, but organisms such as *Aeromonas* spp. may also be included. Procedures to detect and confirm coliforms are used in testing water, foods, dairy products and other materials.¹⁻⁵ Brilliant Green Bile Broth 2% is used to confirm a positive presumptive test result.

Brilliant Green Bile Broth 2% is also referred to as Brilliant Green Bile Broth, Brilliant Green Lactose Broth, Brilliant Green Lactose Bile Broth and Brilliant Green Lactose Bile Broth, 2%.

Principles of the Procedure

Enzymatic Digest of Gelatin is the carbon and nitrogen source used for general growth requirements in Brilliant Green Bile Broth 2%. Oxbile and Brilliant Green inhibit gram-positive bacteria and many gram-negative bacteria, other than coliforms. Lactose is a carbohydrate source. Bacteria that ferment lactose and produce gas are detected.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Lactose	10 g
Oxbile	20 g
Brilliant Green	0.0133 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 40 g of the medium in one liter of purified water until evenly dispersed.
2. Distribute into tubes containing inverted fermentation Durham vials. Autoclave at 121°C for no longer than 15 minutes. To avoid entrapment of bubbles in the fermentation tubes, allow the autoclave to cool at least to 75°C before opening.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green beige.

Prepared Appearance: Prepared medium is emerald green and clear.

Expected Cultural Response: Cultural response in Brilliant Green Bile Broth 2% at 35°C for 18 - 48 hours incubation.

Microorganism	Response	Reactions (Gas)
<i>Enterobacter aerogenes</i> ATCC® 13048	good growth	positive
<i>Enterococcus faecalis</i> ATCC® 29212	marked to complete inhibition	negative
<i>Escherichia coli</i> ATCC® 25922	good growth	positive
<i>Staphylococcus aureus</i> ATCC® 25923	marked to complete inhibition	negative

The organisms listed are the minimum that should be used for quality control testing.

BRILLIANT GREEN AGAR W/ SULFAPYRIDINE (7299)

Intended Use

Brilliant Green Agar w/ Sulfapyridine is used for the selective enrichment of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem. Infection with non-typhi *Salmonella* spp. often causes mild, self-limiting illness.¹ Illness results from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella* spp. Many cases of *Salmonella* related gastroenteritis result from improper handling of poultry products.

Brilliant Green Agar was first described by Kristensen et al.² and later modified by Kauffmann.³ The outstanding selectivity of this medium permits the use of moderately heavy inocula evenly distributed over the surface. The addition of sulfonamides into Brilliant Green Agar further inhibits *Escherichia coli* and *Proteus* spp. Osborne and Stokes used 0.1% Sodium Sulfapyridine to enhance the recovery of *Salmonella* from whole egg and egg yolk.⁴

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are the carbon and nitrogen source used for general growth requirements in this medium. Yeast Extract supplies B-complex vitamins. Lactose and Sucrose are the carbohydrates. In the presence of Phenol Red, a pH indicator, nonlactose and/or nonsucrose-fermenting *Salmonella* spp. will produce red colonies. Sodium Sulfapyridine and Brilliant Green are the selective agents, inhibiting gram-positive organisms and many gram-negative bacteria, except *Salmonella*. Sodium Chloride maintains the osmotic balance. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Lactose	10 g
Sucrose.....	10 g
Brilliant Green	0.0125 g
Phenol Red	0.08 g
Sodium Sulfapyridine	1 g
Agar	20 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 59 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a green tint.

Prepared Appearance: Prepared medium is brown-green, and slightly opalescent, and trace to slightly hazy.

Expected Cultural Response: Cultural response on Brilliant Green Agar w/ Sulfapyridine at 35°C for 18 - 24 hours incubation.

Microorganism	Response	Reaction
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	yellow colonies
<i>Salmonella choleraesuis</i> ATCC® 13076	growth	red colonies
<i>Salmonella typhi</i> ATCC® 19430	partial to complete inhibition	red colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	red colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	----

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for instructions on specific material being tested for *Salmonella*.^{1,5-8}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brilliant Green Agar w/ Sulfapyridine	Code No.	7299A	500 g
		7299B	2 kg
		7299C	10 kg

References

1. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
2. **Kristensen, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, bromthymol blue, bromcresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **Osborne, W. W., and J. L. Stokes.** 1955. The determinations of *Salmonellae* in foods. Ottawa: Food and Drug Laboratories.
5. **U. S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
7. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

Test Procedure

Refer to appropriate references for specific instructions for the material being tested. ¹⁻⁵

1. Subculture from a presumptive positive coliform specimen in Lauryl Sulfate Broth or from typical coliform colonies on Violet Red Bile Agar to tubes of Brilliant Green Bile Broth 2%.
2. Incubate at 35°C for 48 ± 2 hours.
3. Examine for bubbles (gas) in the fermentation tube.

Results

Positive: Bubbles (gas) in fermentation tube.

Negative: No bubbles (gas) in fermentation tube.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brilliant Green Bile Broth 2% Code No.	7119A	500 g
	7119B	2 kg
	7119C	10 kg

References

1. **U. S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRILLIANT GREEN BILE BROTH 2% w/ MUG (7344)

Intended Use

Brilliant Green Bile Broth 2% w/ MUG is used for the detection of coliforms and the fluorogenic detection of *Escherichia coli*.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative anaerobic, gram-negative, non-sporeforming, bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. *Escherichia coli* is a member of the fecal coliform group. The presence of *E. coli* is indicative of fecal contamination.¹

Procedures to detect and confirm the presence of coliforms are used in testing water, foods, dairy products, and other materials.¹⁻⁵ The procedures begin with a presumptive test, and when positive, confirmed using Brilliant Green Bile Broth 2%. The addition of the fluorogenic compound, MUG (4-Methylumbelliferyl-®-D-glucuronide) to this medium permits the rapid detection of *E. coli* when medium is observed for fluorescence using a long-wave UV light source.

Principles of the Procedure

Enzymatic Digest of Gelatin provides the nitrogen and vitamin sources in Brilliant Green Bile Broth 2% w/ MUG. Lactose is the carbohydrate energy source. Ox bile and Brilliant Green inhibit gram-positive bacteria and many gram-negative bacteria, other than coliforms. The substrate MUG (4-Methylumbelliferyl-®-D-glucuronide) produces a blue fluorescence when hydrolyzed by the enzyme ®-glucuronidase, produced by most *E. coli*.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Lactose	10 g
Ox bile	20 g
MUG (4-Methylumbelliferyl®-D-glucuronide).....	0.05 g
Brilliant Green	0.0133 g
Final pH: 7.2 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system and skin.

Directions

1. Dissolve 40 g of the medium in one liter of purified water.
2. Dispense into tubes containing inverted fermentation tubes.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and greenish-beige.

Prepared Appearance: Prepared medium is emerald green and clear.

Expected Cultural Response: Cultural response in Brilliant Green Bile Broth 2% w/ MUG at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions	
		Gas	Fluorescence
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	positive	negative
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	negative	negative
<i>Escherichia coli</i> ATCC® 25922	growth	positive	positive
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	negative	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Follow the methods and procedures for the test being performed using Brilliant Green Bile Broth 2% w/ MUG.

Results

Observe inoculated tube for characteristic growth, gas production, and fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence throughout the tube when exposed to long wave UV light. Non-*E.coli* coliforms may produce gas but do not fluoresce.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Glucuronidase-negative strains of *E. coli* have been encountered⁶⁻⁹ and will be missed by this procedure.
3. Strains of *Salmonella* spp. and *Shigella* spp. that produce glucuronidase may infrequently be encountered.¹⁰ 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.

Packaging

Brilliant Green Bile Broth 2% w/ MUG	Code No.	7344A	500 g
		7344B	2 kg
		7344C	10 kg

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1993. Coliform and other indicator bacteria, p. 247-269. In R. T. Marshall (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington D.C.
4. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler.** 1995. *Escherichia coli* and the coliform bacteria, p. 4.01-4.29. In Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Andrews, W. H.** 1995. Microbial methods, p. 1-119. In Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
6. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of β -D-Glucuronidase-negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.
7. **Hansen, W., and E. Yourassowsky.** 1984. Detection of β -D-Glucuronidase in lactose fermenting members of the family enterobacteriaceae and its presence in bacterial urine cultures. J. Clin. Microbiol. **20**:1177-1179.
8. **Kilian, M. and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. Acta. Pathol. Microbiol. Scand. Sect. B **84**:245-251.
9. **Mates, A., and M. Shaffer.** 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriol. **67**:343-346.
10. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. J. Food Sc. **50**:1736-1746.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRUCELLA AGAR (7120)

Intended Use

Brucella Agar is used for the cultivation of *Brucella* spp. and other fastidious microorganisms.

Product Summary and Explanation

Brucella Agar is prepared according to the APHA formula for Albimi Broth.¹ Brucella Agar is a general purpose medium for the cultivation of *Brucella* spp. and fastidious microorganisms including *Streptococcus pneumoniae*, *Streptococcus viridans*, and *Neisseria meningitidis*.² With the addition of blood, Brucella Agar is used to determine bacterial hemolytic reactions.² Brucella Agar can be used as a base for the isolation of *Campylobacter* spp.²

Brucellosis is a zoonotic disease with a domestic-animal reservoir.³ Transmission by milk, milk products, meat, and direct contact with infected animals are the usual routes of exposure.³

Principles of the Procedure

The nitrogen and carbon source is provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue in Brucella Agar. Yeast Extract is the vitamin source. Dextrose is the carbohydrate. Sodium Chloride maintains the osmotic environment. Sodium Bisulfite is added to enhance growth. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Yeast Extract.....	2 g
Sodium Chloride	5 g
Dextrose.....	1 g
Sodium Bisulfite	0.1 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. *Brucella* spp. are classified as Biosafety Level 3 pathogens. Procedures with live cultures and antigens must be confined to Class II biological safety cabinet (BSC).³
3. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 43 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and yellow beige.

Expected Cultural Response: Cultural response on Brucella Agar at 35°C under 3% CO₂ after 18 - 96 hours incubation.

Microorganism	Response
<i>Brucella abortus</i> ATCC® 4315	growth
<i>Brucella melitensis</i> ATCC® 4309	growth
<i>Brucella suis</i> ATCC® 4314	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the isolation and identification of *Brucella* spp.^{4,5}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brucella Agar	Code No.	7120A	500 g
		7120B	2 kg
		7120C	10 kg

References

1. **Hausler, W. J. (ed.).** 1976. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 110-114. Williams & Wilkins, Baltimore, MD.
3. **Moyer, N. P., and L. A. Holcomb.** 1995. *Brucella*, p. 549-555. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRUCELLA BROTH (7121)

Intended Use

Brucella Broth is used for the cultivation of *Brucella* spp. and other fastidious microorganisms.

Product Summary and Explanation

Brucella Broth is prepared according to the APHA formula for Albimi Broth.¹ Brucella Broth is a general purpose medium for the cultivation of *Brucella* spp. and fastidious microorganisms including, *Streptococcus pneumoniae*, *Streptococcus viridans*, and *Neisseria meningitidis*.² *Brucella* spp. is the causative agent for brucellosis, a zoonotic disease with a domestic-animal reservoir.³ Transmission by milk, milk products, meat, and direct contact with infected animals is the usual route of exposure.³

Brucella Broth is recommended for the isolation of *Brucella* spp. from blood cultures,^{4,5} and specified in standard methods for the examination of food.⁶

Principles of the Procedure

The nitrogen and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue in Brucella Broth. Yeast Extract is the vitamin source in this medium. Dextrose is the carbohydrate source, and Sodium Chloride maintains the osmotic environment. Sodium Bisulfite is added to enhance growth.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Yeast Extract.....	2 g
Sodium Chloride	5 g
Dextrose.....	1 g
Sodium Bisulfite	0.1 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. *Brucella* spp. are classified as Biosafety Level 3 pathogens. Procedures with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).³
3. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 28 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear and light amber.

Expected Cultural Response: Cultural response in Brucella Broth at 35°C under 3% CO₂ after 18 - 72 hours incubation.

Microorganism	Response
<i>Brucella abortus</i> ATCC® 4315	growth
<i>Brucella melitensis</i> ATCC® 4309	growth
<i>Brucella suis</i> ATCC® 4314	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the isolation and identification of *Brucella* spp.^{4,5}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brucella Broth	Code No.	7121A	500 g
		7121B	2 kg
		7121C	10 kg

References

1. **Hausler, W. J. (ed.).** 1976. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 110-114. Williams & Wilkins, Baltimore, MD.
3. **Moyer, N. P., and L. A. Holcomb.** 1995. *Brucella*, p. 549-555. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BUFFERED LISTERIA ENRICHMENT BROTH (7579)

Intended Use

Buffered Listeria Enrichment Broth is used for selective enrichment of *Listeria* spp.

Product Summary and Explanation

Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann,¹ is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.² Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey, frankfurters, coleslaw, pasteurized milk, Mexican style cheese, and pate'.⁴ *Listeria* spp. are ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, and river water.⁵

Buffered Listeria Enrichment Broth, a modification of the formula by Lovett et al.,⁶ was developed after subsequent work concluded that enrichment properties can be improved by increasing the buffering capacity of the medium with the addition of Disodium Phosphate. *Listeria* spp. grow over a pH range of 5.0 - 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, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Soybean Meal, and Yeast Extract provides nitrogen, vitamins, and minerals in Buffered Listeria Enrichment Broth. Dextrose is the carbohydrate source. Sodium Chloride maintains osmotic balance of the medium. Monopotassium Phosphate, Dipotassium Phosphate, and Disodium Phosphate are the buffering agents. Nalidixic Acid inhibits growth of gram-negative organisms. Acriflavin inhibits gram-positive bacteria. Cyclohexamide is used to inhibit growth of saprophytic fungi.

Formula / Liter

Enzymatic Digest of Casein	17 g
Enzymatic Digest of Soybean Meal	3 g
Yeast Extract.....	6 g
Dextrose.....	2.5 g
Sodium Chloride	5 g
Monopotassium Phosphate	1.35 g
Dipotassium Phosphate	2.5 g
Disodium Phosphate.....	9.6 g
Cycloheximide.....	0.05 g
Nalidixic Acid.....	0.04 g
Acriflavin	0.015 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. TOXIC. Harmful by inhalation and if swallowed. Possible risk to unborn child.

Directions

1. Dissolve 47 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and yellow to tan.

Prepared Appearance: Prepared medium is yellow with a green tint.

Expected Cultural Response: Cultural response in Buffered Listeria Enrichment Broth at 30°C after 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	good growth
<i>Staphylococcus aureus</i> ATCC® 25923	suppressed at 18 – 24 hours

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Use recommended laboratory procedures for isolating *Listeria* in food samples.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Buffered Listeria Enrichment Broth	Code No.	7579A	500 g
		7579B	2 kg
		7579C	10 kg

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bact. **29**:407-439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
4. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
5. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
6. **Lovette, J., D. W. Frances, and J. M. Hunt.** 1987. *Listeria monocytogenes* In raw milk: detection, incidence and pathogenicity. J. Food Prot. **50**:188-192.
7. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BUFFERED PEPTONE WATER (BROTH) (7418)

Intended Use

Buffered Peptone Water is used for the non-selective pre-enrichment of *Salmonella* spp. from food.

Product Summary and Explanation

Edel and Kamelmacher¹ found that food preservation techniques involving heat, desiccation, preservatives, high osmotic pressure, or pH changes cause sublethal injury to *Salmonella* spp. Pre-enrichment in a non-selective medium allows for repair of cell damage and facilitates the recovery of *Salmonella*. 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 pre-enrichment period and allows 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 is used in standard methods.⁴

Principles of the Procedure

Peptone is the nitrogen, carbon, vitamin, and mineral sources in Buffered Peptone Water. Sodium Chloride maintains the osmotic balance. Phosphates buffer the medium.

Formula / Liter

Peptone..... 10 g
Sodium Chloride 5 g
Disodium Phosphate..... 3.5 g
Monopotassium Phosphate 1.5 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 20 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium, if necessary.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear and pale yellow.

Expected Cultural Response: Cultural response in Buffered Peptone Water at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	good growth
<i>Salmonella typhimurium</i> ATCC® 14028	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Buffered Peptone Water.

Results

Growth is indicated by turbidity.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Buffered Peptone Water	Code No.	7418A	500 g
		7418B	2 kg
		7418C	10 kg

References

1. **Edel, W., and E. H. Kampelmacher.** 1973. Bull World Hlth. Org. **48**:167-174.
2. **Angelotti, R.** 1963. Microbiological quality of foods. Academic Press, New York.
3. **Sadovski, A. Y.** 1977. J. Food Technol. **12**:85-91.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CAMPY BLOOD FREE SELECTIVE MEDIUM (CCDA) (7527)

Intended Use

Campy Blood Free Selective Medium (CCDA) is used with cefoperazone for the selective isolation of *Campylobacter* spp.

Product Summary and Explanation

Many experts consider *Campylobacter* to be the leading cause of enteric illness in the US.¹ *Campylobacter* spp. can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea.¹ These pathogens are highly infective, and transmitted by contaminated food or water.

Campy Blood Free Selective Medium (CCDA) was described by Bolton et al.² This medium was formulated to replace blood with charcoal, ferrous sulfate, and sodium pyruvate. To improve selectivity, cefoperazone replaced cephalosporin in the original formulation.³ Bolton et al.⁴ recommended incubating inoculated plates at 37°C to improve isolation rates. Yeast and fungal contaminants are inhibited with the addition of amphotericin B.

Campy Blood Free Selective Medium (CCDA) is recommended for food testing.^{1,5}

Principles of the Procedure

Nutrient Broth No. 2 and Casein Acid Hydrolysate provides nitrogen and vitamin sources in this medium. Charcoal absorbs toxic compounds and metabolites. Sodium Desoxycholate and Cefoperazone are selective agents to inhibit enteric flora. Ferrous Sulfate and Sodium Pyruvate are present as oxygen scavengers. Agar is the solidifying agent.

Formula / Liter

Nutrient Broth No. 2	25 g
Charcoal.....	4 g
Casein Acid Hydrolysate	3 g
Sodium Desoxycholate	1 g
Ferrous Sulfate	0.25 g
Sodium Pyruvate.....	0.25 g
Agar	12 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Cefoperazone, 32 mg

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful by inhalation, ingestion, and through skin absorption.

Directions

1. Suspend 45.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and aseptically add 4 mL of a filtered sterilized aqueous solution containing 32 mg of cefoperazone.
5. Mix well and pour into petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and gray-black.

Prepared Appearance: Prepared medium is gray-black.

Expected Cultural Response: Cultural response on Campy Blood Free Selective Medium (CCDA) at 37°C after 18 - 48 hours in an atmosphere consisting of approximately 5 - 6% oxygen, 3 - 10% carbon dioxide, and 84 - 85% nitrogen.

Microorganism	Response
<i>Campylobacter jejuni</i> ATCC® 33291	good growth
<i>Escherichia coli</i> ATCC® 25922	Inhibited

The organisms listed are the minimum that should be used for quality control testing.

Note: Quality Control Laboratory sample was tested with the addition of cefoperazone.

Test Procedure

1. Inoculate the specimen directly onto the surface of the prepared Campy Blood Free Selective Medium (CCDA). If an enrichment broth is required, refer to the appropriate references.^{1,5,6}
2. Streak for isolation.
3. Incubate inoculated plates at 37°C or 42°C in an atmosphere composed of 5 - 6% oxygen, 3 - 10% carbon dioxide and 84 - 85% nitrogen for 24 - 48 hours. Selective temperatures are required for certain *Campylobacter* spp. Refer to appropriate references on the proper temperature for the targeted *Campylobacter* spp.¹

Results¹

Campylobacter colonies are round to irregular with smooth edges. They may have translucent, white colonies to spreading, flat, transparent growth. Some strains appear tan or slightly pink. Normal enteric flora is completely to markedly inhibited.

Typically, *Campylobacter* spp. are oxidase positive and catalase positive. For complete identification of species and biotype, refer to the appropriate procedures for biochemical reactions.^{1,4}

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Campy Blood Free Selective Medium (CCDA)	Code No.	7527A	500 g
		7527B	2 kg
		7527C	10 kg

References

1. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Bolton, F. J., D. N. Hutchinson, and D. Coates.** 1984. J. Clin. Microbiol. **19**:169-171.
3. **Bolton, F. J., and D. N. Hutchinson.** 1984. J. Clin. Pathol. **34**:956-957.
4. **Bolton, F. J., D. N. Hutchinson, and G. Parker.** 1988. Eur. J. Clin. Microbiol. Infect Dis. **7**:155-160.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CAMPY SELECTIVE AGAR BASE (PRESTON) (7443)

Intended Use

Campy Selective Agar Base (Preston) is used with antimicrobics for the selective isolation of *Campylobacter jejuni* and *Campylobacter coli*.

Product Summary and Explanation

Many experts consider *Campylobacter* to be the leading cause of enteric illness in the US.¹ *Campylobacter* spp. can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea.¹ These pathogens are highly infective, and transmitted by contaminated food or water.

Campy Selective Agar Base (Preston) is based on the formulation described by Bolton and Robertson.² This formula, with the addition of the Preston Supplement, was developed to isolate *Campylobacter* spp. from human, animal, and environmental specimens. The Preston formulation demonstrated improved recovery and selectivity of *Campylobacter* spp. in comparative studies with other selective media (Skirrow, Butzler, Blaser and Campy-Blood Agar).³

Principles of the Procedure

Enzymatic Digest of Animal Tissue and Enzymatic Digest of Casein are the nitrogen and vitamin source in this medium. Sodium Chloride provides the osmotic environment, Agar is the solidifying agent. Antimicrobics are added to suppress normal enteric flora, and enhance the growth of *Campylobacter* spp. The addition of 5% lysed horse blood provides essential growth factors.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	10 g
Enzymatic Digest of Casein	10 g
Sodium Chloride	5 g
Agar	12 g

Final pH: 7.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobials / 10 mL

Polymyxin B	5000 IU
Trimethoprim	10 mg
Rifampin	10 mg
Cycloheximide	100 mg

Precautions

1. For In Vitro Diagnostic Use.
2. Follow standard laboratory policies when handling and disposing of contaminated material.
3. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 37 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and aseptically add 5% lysed horse blood and 10 mL of a filtered sterilized aqueous solution containing 5000 IU polymyxin B, 10 mg trimethoprim, 10 mg rifampin, and 100 mg cycloheximide.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium with 5% lysed horse blood is red, clear to trace hazy.

Expected Cultural Response: Cultural response at 37°C after 18 - 48 hours incubation on 5% horse blood plates in an atmosphere consisting of approximately 5 - 6% oxygen, 3 - 10% carbon dioxide and 84 - 85% nitrogen.

Microorganism	Response
<i>Campylobacter jejuni</i> ATCC® 29428	growth
<i>Campylobacter jejuni</i> ATCC® 33291	growth
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited
<i>Proteus mirabilis</i> ATCC® 12453	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate the specimen directly onto the surface of the prepared Campy Selective Agar Base (Preston).
2. Streak for isolation.
3. Incubate inoculated plates at 37°C or 42°C in an atmosphere composed of 5 - 6% oxygen, 3 - 10% carbon dioxide and 84 - 85% nitrogen for 24 - 48 hours. Selective temperatures are required for certain strains of *Campylobacter* spp. Refer to appropriate references on the proper temperature and microaerophilic environment of *Campylobacter* spp.¹

Results¹

Campylobacter colonies are round to irregular with smooth edges. They may have translucent, white colonies to spreading, flat, transparent growth. Some strains appear tan or slightly pink. Normal enteric flora is completely to markedly inhibited.

Typically, *Campylobacter* spp. are oxidase positive and catalase positive. For complete identification of species and biotype, refer to the appropriate procedures for biochemical reactions.^{1,4}

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Campy Selective Agar Base (Preston)	Code No.	7443A	500 g
		7443B	2 kg
		7443C	10 kg

References

1. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Bolton, F. J., and L. Robertson.** 1982. J. Clin. Microbiol. **35**:462-467.
3. **Bolton, F. J., D. Coates, P. M. Hinchliffe, and L. Robertson.** 1983. J. Clin. Pathol. **36**:78-83.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CAMPYLOBACTER ENRICHMENT BROTH (7526)

Intended Use

Campylobacter Enrichment Broth is used with antimicrobics for the selective enrichment of *Campylobacter* spp.

Product Summary and Explanation

Many experts consider *Campylobacter* to be the leading cause of enteric illness in the US.¹ *Campylobacter* spp. can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea.¹ These pathogens are highly infective, and transmitted by contaminated food or water.

Campylobacter spp. are microaerophilic, very small, curved, thin, Gram-negative rods.¹ Microaerophilic organisms have a tendency to be more sensitive to toxic forms of oxygen.² Campylobacter Enrichment Broth, along with nutritional ingredients, contains compounds which enhance the aerotolerance of microaerophilic bacteria by suppressing the toxic form of oxygen.² Campylobacter Enrichment Broth is recommended in food testing.¹

Principles of the Procedure

Enzymatic Digest of Animal Tissue, Lactalbumin Hydrolysate, and Yeast Extract provide nitrogen, carbon, amino acids, and vitamins in Campylobacter Enrichment Broth. Hemin and Lysed Horse Blood provide essential growth factors. Sodium Chloride maintains the osmotic balance of the medium. Sodium Pyruvate, Sodium Metabisulphite, and Sodium Carbonate increase the aerotolerance of *Campylobacter* spp. by acting as oxygen scavengers. The addition of cefoperazone, cycloheximide, trimethoprim, and vancomycin are selective agents for heavily contaminated samples.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	10 g
Lactalbumin Hydrolysate.....	5 g
Yeast Extract.....	5 g
Sodium Chloride	5 g
Hemin.....	0.01 g
Sodium Pyruvate.....	0.5 g
α -Ketoglutamic Acid.....	1 g
Sodium Metabisulphite.....	0.5 g
Sodium Carbonate	0.6 g

Final pH: 7.4 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobial / 10 mL of Ethanol

Cefoperazone	20 mg
Cycloheximide	50 mg
Trimethoprim	20 mg
Vancomycin	20 mg

Enrichment

Lysed Horse Blood	50 mL

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful if swallowed, inhaled, ingested, or absorbed through skin.

Directions

1. Dissolve 27.6 g of the medium in one liter of purified water.
2. Allow powder to soak for 10 minutes.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and aseptically add 50 mL of lysed horse blood and 10 mL of ethanol containing 20 mg of cefoperazone, 50 mg of cycloheximide, 20 mg trimethoprim, and 20 mg vancomycin.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and amber.

Expected Cultural Response: Cultural response, after incubation in *Campylobacter* Enrichment Broth, on Blood Agar Base No. 2 after 24 - 48 hour incubation at 35°C.

Microorganism	Response	
	w/ Antibiotic	w/o Antibiotic
<i>Campylobacter jejuni</i> ATCC® 29428	good growth	good growth
<i>Campylobacter jejuni</i> ATCC® 33291	good growth	good growth
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	good growth
<i>Escherichia coli</i> ATCC® 25922	inhibited	good growth
<i>Proteus mirabilis</i> ATCC® 12453	inhibited	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to the appropriate procedure for the material being testing on the isolation of *Campylobacter* spp. Refer to standard methods for food testing.¹

Results¹

Campylobacter colonies are round to irregular with smooth edges. They may have translucent, white colonies to spreading, flat, transparent growth. Some strains appear tan or slightly pink. Normal enteric flora is completely to markedly inhibited. Typically, *Campylobacter* spp. are oxidase positive and catalase positive. For complete identification of species and biotype, refer to the appropriate procedures for biochemical reactions.^{1,3}

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Campylobacter Enrichment Broth	Code No.	7526A	500 g
		7526B	2 kg
		7526C	10 kg

References

1. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed., AOAC International, Gaithersburg, MD.
2. **George, H. A., P. S. Hoffman, and N. R. Krieg.** 1978. J. Clin. Micro. 8:36-41.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BEEF EXTRACT POWDER (7228)

Intended Use

Beef Extract Powder is a dehydrated extract of bovine tissue for use in preparing microbiological culture media.

Product Summary and Explanation

Beef Extract Powder is prepared and standardized for use in microbiological culture media, where it is generally used to replace infusion of meat. Culture media containing Beef Extract Powder are recommended for use in bacteriological examination of water, milk, and other materials, where uniform composition of media is important. Beef Extract Powder is relied upon for biochemical studies, particularly fermentation reactions because of its independence from fermentable substances. Several media containing Beef Extract Powder are recommended in standard methods for multiple application.¹⁻³

Principles of the Procedure

Beef Extract Powder provides nitrogen, amino acids, vitamins, and carbon. Beef Extract Powder is usually employed in concentrations of 0.3% in culture media. Concentrations may vary slightly according to the requirements of individual formulas, but do not often exceed 0.5%.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and dark beige to tan.

Prepared Appearance (2% wt/vol): Prepared medium is clear, amber with no or a light precipitate.

pH (2% Solution at 25°C): 6.5 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35° C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to excellent growth

Test Procedure

Refer to appropriate references for specific procedures using Beef Extract Powder.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Beef Extract Powder at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Beef Extract Powder should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to Beef Extract Powder in its intact container when stored as directed.

Packaging

Beef Extract Powder	Code No.	7228A	500 g
		7228B	2 kg
		7228C	10 kg

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BETA-SSA AGAR (7336)

Intended Use

Beta-SSA Agar is used with blood for the selective isolation of group A streptococci.

Product Summary and Explanation

Group A streptococcal infections are the most common cause of bacterial pharyngitis in children 5 to 10 years old.¹ Beta-SSA Agar is a highly selective agar developed for the isolation of group A beta-hemolytic streptococci. The selective agents in the medium inhibit gram-negative bacteria and most Gram-positive bacteria, although some strains of group B beta-hemolytic streptococci may grow. Beta-SSA Agar is supplemented with 5% sheep blood to detect hemolytic patterns of streptococci.

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal. Sodium Chloride maintains the osmotic balance of the medium. Selective Agents inhibit gram-negative bacteria and most gram-positive bacteria. Agar is the solidifying agent.

In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β -hemolytic streptococci.² Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and type of basal medium used.¹

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Selective Agents	0.0462 g
Agar	15 g

Final pH: 7.3 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium without blood is beige and trace to slightly hazy. With 5% sheep blood the medium is red and opaque.

Expected Cultural Response: Cultural response on Beta-SSA Agar supplemented with 5% sheep blood at 35°C after 18 - 24 hours incubation.

<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---
<i>Streptococcus pneumoniae</i> ATCC® 6305	inhibited	---
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis (clear zone w/ Bacitracin disk)

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.¹
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results³

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Atmosphere of incubation is known to influence hemolytic reactions of beta-hemolytic streptococci.¹ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Packaging

Beta-SSA Agar	Code No.	7336A	500 g
		7336B	2 kg
		7336C	10 kg

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
2. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
3. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BIGGY AGAR (7191)

Intended Use

BIGGY Agar is used for the isolation and differentiation of *Candida* spp.

Product Summary and Explanation

BIGGY Agar is a modification of the formula described by Nickerson.^{1,2} BIGGY Agar, an abbreviation for Bismuth Glucose Glycine Yeast Agar, is also referred to as Nickerson Agar and Nickerson Candida Selective Agar.

BIGGY Agar was developed while studying sulfite reduction by *Candida* spp. Nickerson found that *Candida albicans* can be differentiated from other *Candida* spp. on this medium based on colony morphology.

Candidiasis, the most frequently encountered opportunistic fungal infection is usually caused by *Candida albicans*.³ *Candida tropicalis* and *Candida (Torulopsis) glabrata* infections occur less frequently.³ *Candida* spp. are present in clinical specimens as a result of environmental contamination, colonization, or an actual disease process.⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon, source is provided by Yeast Extract in BIGGY Agar. Glycine is used to stimulate growth. Dextrose is the carbohydrate source. *Candida* spp. reduce Bismuth Ammonium Citrate, and colonies become brown to black in color. Bismuth Ammonium Citrate and Sodium Sulfite are selective agents against bacteria, often present as normal flora. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	1 g
Glycine	10 g
Dextrose.....	10 g
Bismuth Ammonium Citrate	5 g
Sodium Sulfite.....	3 g
Agar	16 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful by inhalation, ingestion, and skin contact.

Directions

1. Suspend 45 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to moderately hazy, and grey-white in color.

Expected Cultural Response: Cultural response at 30°C after 18 - 72 hours incubation.

Microorganism	Response	Reactions
<i>Candida albicans</i> ATCC® 10231	growth	brown-black, no diffusion w/ mycelial fringe
<i>Candida tropicalis</i> ATCC® 750	growth	dark brown w/ black center and black diffusion around colonies
<i>Escherichia coli</i> ATCC® 25922	inhibited	--
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and identification of yeast spp.^{3,4}

Results

Colony morphology, according to Nickerson,² after 48 hours of incubation on BIGGY Agar:

<i>C. albicans</i>	Intensely brown-black colonies with slight mycelial fringe, medium sized, no diffusion.
<i>C. tropicalis</i>	Discrete dark brown colonies with black centers, and sheen, medium sized, diffuse blackening of the surrounding medium after 72 hours of incubation.
<i>C. pseudotropicalis</i>	Large, dark red-brown colonies, flat, with slight mycelial fringe.
<i>C. krusei</i>	Large, flat, wrinkled colonies with silver-black top, brown edge, and yellow halo.
<i>C. parakrusei</i>	Medium size, flat, wrinkled colonies with red-brown color, and yellow mycelial fringe.
<i>C. stellatoidea</i>	Medium size, flat, dark brown colonies, very light mycelial fringe.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Pigmented bacterial and yeast-like fungi are usually inhibited on BIGGY Agar. They can be differentiated by microscopic examination. Dermatophytes and molds seldom appear, and are easily recognized by development of aerial mycelia.⁵
3. Further growth characteristic and biochemical tests are needed to differentiate yeasts, particularly identification of *Candida* spp.⁵
4. BIGGY Agar should be inoculated when the medium is freshly prepared.^{1,2}
5. Do not prepare slants of BIGGY Agar, the reactions are unsatisfactory.^{1,2}

Packaging

BIGGY Agar	Code No.	7191A	500 g
		7191B	2 kg
		7191C	10 kg

References

1. **Nickerson, W. J.** 1947. Biology of pathogenic fungi. The Chronica Botanica Co., Waltham, MA.
2. **Nickerson, W. J.** 1953. Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulfite by species of *Candida*. J. Infect. Dis. **93**:43.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
4. **Warren, N. G., and K. C. Hazen.** 1995. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 723-737. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 65-68. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BILE ESCULIN AGAR (7249)

Intended Use

Bile Esculin Agar is used for the selective isolation and differentiation of group D streptococci.

Product Summary and Explanation

Bile Esculin Agar is based on the formulation described by Swan and further evaluated by Facklam and Moody.^{1,2} Rochaix first noted the value of esculin hydrolysis in the identification of enterococci.³ Meyer and Schonfeld added bile to the esculin medium and demonstrated 61 of 62 enterococci strains were able to grow and hydrolyze esculin, while other streptococci could not.⁴

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described as group D streptococci, in the genus *Enterococcus*.⁵ The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci. Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping,¹ and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction. Facklam and Moody found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.²

Bile Esculin Agar is in standard procedures for the microbiological examination of food products.⁶⁻⁸

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxbile is used to inhibit gram-positive bacteria other than enterococci. Beef Extract and Enzymatic Digest of Gelatin are the carbon and nitrogen sources used for general growth requirements in Bile Esculin Agar. Agar is the solidifying agent.

Formula / Liter

Beef Extract	11 g
Enzymatic Digest of Gelatin	34.5 g
Esculin	1 g
Oxbile	2 g
Ferric Ammonium Citrate	0.5 g
Agar	15 g

Final pH: 6.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 64 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to dark beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, opalescent, and grey-yellow.

Expected Cultural Response: Cultural response on Bile Esculin Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction Esculin Hydrolysis
<i>Enterococcus faecalis</i> ATCC® 19433	growth	+ (black colonies)
<i>Enterococcus faecalis</i> ATCC® 29212	growth	+ (black colonies)
<i>Enterococcus faecalis</i> ATCC® 33186	growth	+ (black colonies)
<i>Escherichia coli</i> ATCC® 25922	growth	-
<i>Streptococcus pyogenes</i> ATCC® 19615	inhibited	-

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for instructions on specific material being tested for group D streptococci.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Bile Esculin Agar	Code No.	7249A	500 g
		7249B	2 kg
		7249C	10 kg

References

1. **Swan, A.** 1954. The use of bile-esculin medium and of Moxed's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. **7**:160.
2. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. **20**:245.
3. **Rochaix, A.** 1924. Milieux a leculine pour le diagnostid differentiel des bacteries du grojps strepto-entero-pneumocoque. Comt. Rend. Soc. Biol. **90**:771-772.
4. **Meyer, K., and H. Schönfeld.** 1926. Über die Unterscheidung des Enterococcus vom Streptococcus viridans und die Beziehung beider zum Strptoccus lactis. Zentralb. Bakteriell Parasitenkd. Infektionskr. Hyg. Abt. I orig. **99**:402-416.
5. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. **10**:1-19.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, MD.
8. **Marshall, R. T. (ed.).** 1992. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BILE ESCULIN AZIDE AGAR (7133)

Intended Use

Bile Esculin Azide Agar is used for the selective isolation and differentiation of group D streptococci.

Product Summary and Explanation

Bile Esculin Azide Agar is a modification of the medium reported by Isenberg and Isenberg, Goldberg, and Sampson.^{1,2} This formula modifies Bile Esculin Agar by adding sodium azide and reducing the concentration of bile. The revised medium is more selective, but still provides rapid growth and efficient recovery of group D streptococci.

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described group D streptococci, in the genus *Enterococcus*.³ The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci. Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping, and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.⁴ Facklam and Moody found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.⁵

Sabbaj, Sutter, and Finegold evaluated selective media for selectivity, sensitivity, detection, and enumeration of presumptive group D streptococci from human feces.⁶ Bile Esculin Azide Agar selected for *S. bovis*, displayed earlier distinctive reactions, and eliminated the requirement for special incubation temperatures.

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxbile is used to inhibit gram-positive bacteria other enterococci, while Sodium Azide inhibits gram-negative bacteria. Enzymatic Digest of Casein and Yeast Enriched Meat Peptone are the carbon, nitrogen, and vitamin sources used for general growth requirements in Bile Esculin Agar. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate acts as a preservative. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	25 g
Yeast Enriched Meat Peptone	9.5 g
Oxbile.....	1 g
Sodium Chloride	5 g
Sodium Citrate	1 g
Ferric Ammonium Citrate.....	0.5 g
Esculin	1 g
Sodium Azide	0.25 g
Agar	14 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL BY INHALATION AND IF SWALLOWED. Avoid contact with skin and eyes.

Directions

1. Suspend 56 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to dark beige.

Prepared Appearance: Prepared medium is grey to green-yellow, opalescent, and trace to slightly hazy.

Expected Cultural Response: Cultural response on Bile Esculin Azide Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction Esculin Hydrolysis
<i>Enterococcus faecalis</i> ATCC® 29212	growth	+ (blackening around colonies)
<i>Escherichia coli</i> ATCC® 25922	inhibited	-
<i>Streptococcus pyogenes</i> ATCC® 19615	partial to complete inhibition	- (colorless colonies)

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for instructions on specific material being tested for group D streptococci.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Bile Esculin Azide Agar	Code No.	7133A	500 g
		7133B	2 kg
		7133C	10 kg

References

1. **Isenberg, H. D.** 1970. Clin. Lab. Forum.
2. **Isenberg, H. D., D. Goldberg, and J. Sampson.** 1970. Laboratory studies with a selective enterococcus medium. Appl. Microbiol. **20**:433.
3. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. **10**:1-19.
4. **Swan, A.** 1954. The use of bile-esculin medium and of Moxed's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. **7**:160.
5. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. **20**:245.
6. **Sabbaj, J., V. L. Sutter, and S. M. Finegold.** 1971. Comparison of selective media for isolation of presumptive group D streptococci from human feces. Appl. Microbiol. **22**:1008.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BILE SALTS MIXTURE #3 (7230)

Intended Use

Bile Salts Mixture #3 is a mixture of bile salts for use in preparing microbiological culture media.

Product Summary and Explanation

Bile Salts Mixture #3 is a mixture of sodium cholate and sodium deoxycholate. Its primary use is as a selective agent for the isolation of gram-negative microorganisms, inhibiting gram-positive cocci. Bile Salts Mixture #3 is prepared especially for use in MacConkey Agar and Violet Red Bile Agar. Bile Salts Mixture #3 is soluble in distilled or deionized water and neutral to slightly alkaline in pH. It is used in preparing culture media without pre-filtration or adjustment of reaction. Several media containing Bile Salts Mixture #3 are recommended in standard methods for multiple applications.¹⁻³

Principles of the Procedure

Bile is derived from the liver. Bile Salts Mixture #3 contains bile extract standardized to provide inhibitory properties for selective media. Bile Salts Mixture #3 inhibits the growth of gram-positive organisms and spore-forming bacilli without affecting the development of gram-negative enteric bacilli.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and white in color.

Prepared Appearance (2.0 % wt/vol): Prepared medium is clear, colorless to light amber without a precipitate.

pH (2% Solution at 25°C): 7.8 - 8.6

Expected Cultural Response: Cultural response in MacConkey Agar after incubation at 35°C for 18 - 24 hours.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth, pink to red colonies surrounded by a zone of precipitated bile
<i>Staphylococcus aureus</i> ATCC 25923	complete inhibition

Test Procedure

Refer to appropriate references for specific procedures using Bile Salts Mixture #3.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Bile Salts Mixture #3 at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Bile Salts Mixture #3 should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Bile Salts Mixture #3 in its intact container when stored as directed.

Packaging**Bile Salts Mixture #3****Code No.****7230A****500 g****7230B****2 kg****7230C****10 kg****References**

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BISMUTH SULFITE AGAR (7113)

Intended Use

Bismuth Sulfite Agar is used for the selective isolation of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and/or neurological damage.¹ Salmonellosis can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella*. U. S. federal guidelines require various poultry products to be routinely monitored before distribution for human consumption.

Bismuth Sulfite Agar is a modification of Wilson and Blair formula.²⁻⁴ The typhoid organism grows luxuriantly on the medium, forming characteristic black colonies. Gram-positive bacteria and coliforms are inhibited on Bismuth Sulfite Agar. The inhibitory action of Bismuth Sulfite Agar permits the use of a large inoculum, increasing the possibility of recovering pathogens that may be present in small numbers. Bismuth Sulfite Agar is generally accepted for routine detection of most *Salmonella* spp. Bismuth Sulfite Agar is used for the isolation of *S. typhi* and other *Salmonella* spp. from food, feces, urine, sewage, and other infectious materials. Bismuth Sulfite Agar is a standard methods medium for industrial applications and the clinical environment.⁵⁻⁹

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide sources of nitrogen, carbon, and vitamins required for organism growth. Dextrose is the carbohydrate present in Bismuth Sulfite Agar. Disodium Phosphate is the buffering agent. Bismuth Sulfite Indicator and Brilliant Green are complementary, inhibiting gram-positive bacteria and coliforms, allowing *Salmonella* spp. to grow. Ferrous Sulfate is used for H₂S production. When H₂S is present, the iron in the formula is precipitated, and positive cultures produce the characteristic brown to black color with metallic sheen. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Beef Extract	5 g
Dextrose.....	5 g
Disodium Phosphate.....	4 g
Ferrous Sulfate	0.3 g
Bismuth Sulfite Indicator	8 g
Brilliant Green	0.025 g
Agar	20 g

Final pH: 7.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful by inhalation, ingestion, and skin contact.

Directions

1. Suspend 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute.
3. Mix thoroughly to obtain a uniform suspension prior to dispensing.
4. Prepared plates may be used the same day as prepared.
5. For increased selectivity, current references suggest that prepared BSA plates be stored overnight in the dark at room temperature.⁸

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green-beige.

Prepared Appearance: Prepared medium at 45 - 50°C is green-yellow and opaque.

Expected Cultural Response: Cultural response on Bismuth Sulfite Agar at 35°C after 40 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	---
<i>Escherichia coli</i> ATCC® 25922	partially inhibited	brown-green colonies
<i>Salmonella typhimurium</i> ATCC® 19430	growth	black colonies with metallic sheen
<i>Shigella flexneri</i> ATCC® 12022	growth	brown colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For isolation of *Salmonella typhi* and other *Salmonella* spp. consult appropriate references.

Results

Typical *S. typhi* surface colonies are black, surrounded by black or brown-black zone. This zone may be several times the size of the colony. Other strains of *Salmonella* produce black to green colonies with little or no darkening of surrounding medium. *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.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Streak for well isolated colonies. In heavy growth areas, *S. typhi* appears light green and may be misinterpreted as negative for *S. typhi*.¹⁰
2. *S. typhi* and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. However, *S. arizonae* is usually inhibited.¹⁰ 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.¹⁰
3. Do not autoclave medium. Heating Bismuth Sulfite Agar for a period longer than necessary may destroy selectivity properties.

Packaging

Bismuth Sulfite Agar	Code No.	7113A	500 g
		7113B	2 kg
		7113C	10 kg

References

1. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. **Wilson, W. J., and E. M. Blair.** 1926. A combination of bismuth and sodium sulphite affording an enrichment and selective medium for the typhoid-paratyphoid groups of bacteria. J. Pathol. Bacteriol. **29**:310.
3. **Wilson, W. J., and E. M. Blair.** 1927. Use of a glucose bismuth sulphite iron medium for the isolation of *B. typhosus* and *B. proteus*. J. Hyg. **26**:374-391.
4. **Wilson, W. J., and E. M. Blair.** 1931. Further experience of the bismuth sulphite media in the isolation of *B. typhosus* and *B. proteus*. J. Hyg. **31**:138-161.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. **Vanderzant, C., and D.F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
8. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. In FDA Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
9. **Cunniff, P. (ed.).** Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
10. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BLOOD AGAR BASE NO. 2 (7266)

Intended Use

Blood Agar Base NO. 2 is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Blood agar bases are 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, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.¹ Blood Agar Base NO. 2 is a nutritionally rich medium for maximum recovery of fastidious microorganisms. Blood Agar Base media are specified in standard method procedures for food testing.²⁻⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Animal Tissue, Enzymatic Digest of Casein, Liver Digest, and Yeast Extract in Blood Agar Base NO. 2. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	7.5 g
Enzymatic Digest of Animal Tissue.....	7.5 g
Liver Digest	2.5 g
Yeast Extract.....	5 g
Sodium Chloride	5 g
Agar	12 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 39.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light amber, and trace to slightly hazy. With 5% sheep blood, medium is cherry red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Based No. 2 with 5% sheep blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	-
<i>Neisseria meningitidis</i> ATCC® 13090	growth	-
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for oxygen-stable and oxygen-labile streptolysins.⁵
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁶

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (α') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance or has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁵
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.⁵ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%), in accordance with established laboratory procedures.

Packaging

Blood Agar Base NO. 2	Code No.	7266A	500 g
		7266B	2 kg
		7266C	10 kg

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
4. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
6. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BLOOD AGAR BASE w/ LOW pH (7354)

Intended Use

Blood Agar Base w/ LOW pH is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Blood agar bases are 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, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.¹ Lowering the pH in Blood Agar Base w/ LOW pH is used to enhance hemolytic reactions of streptococci.² Blood Agar Base media are specified in standard method procedures for food testing.³⁻⁵

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Heart Infusion Solids and Enzymatic Digest of Animal Tissue in Blood Agar Base w/ LOW pH. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Heart Infusion Solids 10 g
Enzymatic Digest of Animal Tissue 10 g
Sodium Chloride 5 g
Agar 15 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light amber, clear to slightly hazy, without significant precipitate. With 5% sheep blood, medium is cherry red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Base w/ LOW pH with 5% sheep blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis-
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.⁶
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁷

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (α') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁶
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.⁶ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%).

Packaging

Blood Agar Base w/ LOW pH	Code No.	7354A	500 g
		7354B	2 kg
		7354C	10 kg

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Norton, J. F.** 1932. Bacteriology of pus. J. Lab. Clin. Med. **17**:558-564.
3. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
5. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
7. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BLOOD AGAR BASE, IMPROVED (7268)

Intended Use

Blood Agar Base, Improved is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Blood agar bases are 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, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.¹ In Blood Agar Base, Improved, the formula was modified slightly to enhance organism growth and neutralize any toxic metabolites. Blood Agar Base media are specified in standard method procedures for food testing.²⁻⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue in Blood Agar Base, Improved. Yeast Extract is a vitamin source. Corn Starch is added to ensure any toxic metabolites produced are absorbed, and enhance organism growth.⁵ Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Animal Tissue.....	4 g
Yeast Extract.....	2 g
Corn Starch.....	1 g
Sodium Chloride	5 g
Agar	14 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light amber and hazy. With 5% sheep blood, medium is cherry red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Base, Improved with 5% sheep blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	---
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.⁶
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁷

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (α') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁶
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.⁶ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%).

Packaging

Blood Agar Base, Improved	Code No.	7268A	500 g
		7268B	2 kg
		7268C	10 kg

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
4. **Greenberg, A. E., L. S. Clesceri, and A.D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 141-143. Williams & Wilkins, Baltimore, MD.
6. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
7. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BLOOD AGAR BASE, pH 7.4 (7114)

Intended Use

Blood Agar Base, pH 7.4 is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Blood agar bases are 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, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.¹ Blood Agar Base media are specified in standard method procedures for food testing.²⁻⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Heart Muscle Infusion and Enzymatic Digest of Animal Tissue in Blood Agar Base, pH 7.4. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Heart Muscle Infusion	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Sodium Chloride	5 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light amber, trace to slightly hazy. With 5% sheep blood, medium is cherry red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Base, pH 7.4 with 5% sheep blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	---
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.⁵
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁶

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (α') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁵
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.⁵ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%).

Packaging

Blood Agar Base, pH 7.4	Code No.	7114A	500 g
		7114B	2 kg
		7114C	10 kg

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
4. **Greenberg, A. E., L. S. Clesceri, and A.D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
6. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRAIN-HEART INFUSION AGAR (7115)

Intended Use

Brain-Heart Infusion Agar is used for the cultivation of a wide variety of fastidious organisms.

Product Summary and Explanation

Rosenow prepared a rich medium for culturing streptococci by combining dextrose broth and brain tissue.¹ Hayden modified the original formula while working with dental pathogens.² The current formula is a modification of Rosenow and Hayden, using dehydrated infusions of pork brain and pork heart tissue.

Brain-Heart Infusion Agar can be supplemented with antibiotics, varying amounts of sodium chloride, yeast extract and serum to provide a rich medium for bacteria, yeast, and pathogenic fungi.³ Brain-Heart Infusion Agar (BHI Agar), is specified in many references for food and water testing.⁴⁻⁶ Standard Methods for the Examination of Water and Wastewater recommends Brain-Heart Infusion media in tests for the verification of fecal streptococci.⁷

Principles of the Procedure

The nitrogen, vitamin, and carbon source is provided by Brain Heart Infusion, Enzymatic Digest of Animal Tissue, and Enzymatic Digest of Casein in BHI Agar. Dextrose is the carbohydrate source, and Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent.

Formula / Liter

Brain Heart Infusion (Solids).....	8 g
Enzymatic Digest of Animal Tissue.....	5 g
Enzymatic Digest of Casein	16 g
Dextrose.....	2 g
Sodium Chloride	5 g
Disodium Phosphate.....	2.5 g
Agar	13.5 g

Final pH 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, and light to medium amber.

Expected Cultural Response: Cultural response on Brain-Heart Infusion Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Candida albicans</i> ATCC® 10231	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Brain-Heart Infusion Agar.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brain-Heart Infusion Agar	Code No.	7115A	500 g
		7115B	2 kg
		7115C	10 kg

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Research **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 147-153, CRC Press, Boca Raton, FL.
4. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
5. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.
7. **Greenberg, A. E., L. S. Clesceri, and A.D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRAIN-HEART INFUSION BROTH (7116)

Intended Use

Brain-Heart Infusion Broth is used for the cultivation of a wide variety of fastidious organisms.

Product Summary and Explanation

Rosenow¹ prepared a rich medium for culturing streptococci by combining dextrose broth and brain tissue. Hayden² modified the original formula while working with dental pathogens. The current formula is a modification of Rosenow¹ and Hayden², using dehydrated infusions of porcine brain and heart tissue.

Brain-Heart Infusion Broth can be supplemented with antibiotics, varying amounts of sodium chloride, yeast extract, and serum to provide a rich medium for bacteria, yeasts and pathogenic fungi.³ The addition of 0.1% agar can be used to lower oxygen tension, providing an atmosphere to support the growth of aerobic, microaerophilic, and obligate anaerobic microorganisms.

Brain-Heart Infusion Broth, abbreviated as BHI, is specified in many references for food and water testing.⁴⁻⁷ NCCLS, National Committee for Clinical Laboratory Standards, cites Brain-Heart Infusion Broth for preparing the inoculum used in antimicrobial susceptibility tests.⁸

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Brain-Heart Infusion and Enzymatic Digest of Gelatin in BHI Broth. Dextrose is the carbohydrate source, and Sodium Chloride maintains the osmotic environment. Disodium Phosphate is the buffering agent in this medium.

Formula / Liter

Brain Heart Infusion 17.5 g
Enzymatic Digest of Gelatin 10 g
Dextrose..... 2 g
Sodium Chloride 5 g
Disodium Phosphate..... 2.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 37 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared broth is clear, with and without a minor precipitate, and light to medium amber in color.

Expected Cultural Response: Cultural response at 35°C for 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Brain-Heart Infusion Broth.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brain-Heart Infusion Broth	Code No.	7116A	500 g
		7116B	2 kg
		7116C	10 kg

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Research **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 147-153, CRC Press, Boca Raton, FL.
4. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
5. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.
7. **Greenberg, A. E., L. S. Clesceri, and A.D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
8. **National Committee for Clinical Laboratory Standards.** 1994. M11-A3, Vol. 13, No. 26, Methods for antimicrobial susceptibility testing of anaerobic bacteria. National Committee for Clinical Laboratory Standards, Villanova, PA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRAIN-HEART INFUSION SOLIDS (7262)

Intended Use

Brain-Heart Infusion Solids is dehydrated infusion of porcine brains and hearts for use in preparing microbiological culture media.

Product Summary and Explanation

Rosenow devised an excellent medium for culturing streptococci by supplementing Dextrose Broth with bovine brain tissue.¹ Hayden, revising Rosenow's procedure by adding crushed marble to the medium, reported favorable growth of organisms from dental pathogens. Brain-Heart Infusion Solids was developed as an alternative to bovine based Brain Heart Infusion containing media, replacing calf brain and beef heart with porcine brains and hearts. Brain-Heart Infusion Solids was developed for pharmaceutical and vaccine production, and can replace traditional bovine based BHI media in most applications.

The nutritionally rich formula of Brain-Heart Infusion Solids 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

Brain-Heart Infusion Solids provides nitrogen, amino acids, and vitamins in microbiological culture media. Brain-Heart Infusion Solids is processed from large volumes of raw material, retaining all the nutritive and growth stimulating properties of fresh tissue.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogenous, free-flowing beige.

Prepared Appearance (2% wt/vol): Prepared medium is clear, amber with no or a light precipitate.

pH (2% Solution at 25°C): 6.5 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to good growth

Test Procedure

Refer to appropriate references for specific procedures using Brain-Heart Infusion Solids.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Brain-Heart Infusion Solids at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Brain-Heart Infusion Solids should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Brain-Heart Infusion Solids in its intact container when stored as directed.

Packaging

Brain-Heart Infusion Solids	Code No.	7262A	500 g
		7262B	2 kg
		7262C	10 kg

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Res. **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Cunniff, P. (ed.).** 1995. Official methods of analysis, AOAC International, 16th ed. AOAC International, Arlington, VA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRAIN-HEART INFUSION w/o DEXTROSE (7193)

Intended Use

Brain-Heart Infusion w/o Dextrose is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Rosenow¹ prepared a rich medium for culturing streptococci by combining dextrose broth and brain tissue. Hayden² modified the original formula while working with dental pathogens. The current formula is a modification of Rosenow¹ and Hayden,² using dehydrated infusions of pork brain and heart tissue.

Brain-Heart Infusion w/o Dextrose is a basal medium used with added carbohydrates for fermentation studies, or supplemented with blood. Brain-Heart Infusion media is specified in standard methods.³⁻⁵

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Brain-Heart Infusion and Enzymatic Digest of Gelatin. Sodium Chloride maintains the osmotic environment. Dipotassium Phosphate is the buffering agent in this medium.

Formula / Liter

Brain-Heart Infusion (dehydrated).....	17.5 g
Enzymatic Digest of Gelatin	10 g
Sodium Chloride	5 g
Dipotassium Phosphate	2.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 35 g of the medium in one liter of purified water until evenly dissolved.
2. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear with and without a minor precipitate and light to medium amber.

Expected Cultural Response: Cultural response in Brain-Heart Infusion w/o Dextrose at 35°C after 18 - 96 hours incubation.

Microorganism	Response
<i>Haemophilus parasuis</i> ATCC® 19417	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Brain-Heart Infusion w/o Dextrose or carbohydrate fermentation studies.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brain-Heart Infusion w/o Dextrose	Code No.	7193A	500 g
		7193B	2 kg
		7193C	10 kg

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Research **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
4. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRILLIANT GREEN AGAR (7117)

Intended Use

Brilliant Green Agar is used for the selective isolation of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *Salmonella typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and/or neurological damage.¹ Infection can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella*. U. S. federal guidelines require various poultry products routinely monitored before distribution for human consumption.

Kristensen, Lester, and Jurgens first described the use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella*.² The report described the medium as useful for the differentiation of "paratyphoid B" from other intestinal gram-negative bacilli.² Kaufmann modified their formula and used Brilliant Green Agar in addition to Tetrathionate Broth for the isolation of *Salmonella* from stool specimens.³

Brilliant Green Agar is recommended for use in testing clinical specimens.^{1,4} The outstanding selectivity of this medium permits use of moderately heavy inocula, which should be evenly distributed over the surface. Brilliant Green Agar is used in the microbial limits test.⁵ Brilliant Green Agar supplemented with novobiocin is used in food testing.⁶

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide sources of nitrogen, amino acids, and carbon. Yeast Extract supplies vitamins required for organism growth. Sodium Chloride maintains the osmotic balance of the medium. Lactose and Sucrose are the carbohydrates in the medium. Brilliant Green inhibits gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Phenol Red is the pH indicator and turns the medium yellow with the formation of acid when lactose and/or sucrose is fermented. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Lactose	10 g
Sucrose	10 g
Brilliant Green	0.0125 g
Phenol Red	0.08 g
Agar	20 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 58 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a green tint.

Prepared Appearance: Prepared medium is brown-green, slightly opalescent, and may be trace to slightly hazy.

Expected Cultural Response: Cultural response on Brilliant Green Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	partial inhibition	green colonies
<i>Salmonella choleraesuis</i> ATCC® 13076	growth	pink colonies
<i>Salmonella typhi</i> ATCC® 19430	partial inhibition	pink colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	pink colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For isolation of *Salmonella* from clinical specimens, inoculate fecal specimens and rectal swabs on the first quadrant of Brilliant Green Agar and streak for isolation. Incubate plates at 35°C. Examine plates after 18 – 24 hours for colonies with characteristic morphologies associated with *Salmonella* spp. Refer to appropriate references or standard methods for other applications.

Results

Typical *Salmonella* spp. colonies are opaque and pink. The few lactose and/or sucrose fermenting Organisms that grow are readily differentiated due to formation of green colonies. Brilliant Green Agar is not suitable for the isolation of *S. typhi* or *Shigella* spp., although some strains of *S. typhi* may grow.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Colonies of *Salmonella* spp. can be red, pink, or white depending on length of incubation and strain.⁷
2. Medium is normally orange-brown, however after incubation it can turn bright red and return to normal color at room temperature.⁷
3. Taylor showed that slow lactose fermenters, *Proteus*, *Citrobacter*, and *Pseudomonas* may grow on Brilliant Green Agar as red colonies.⁸
4. Other primary plating medium such as MacConkey Agar should be used when testing for intestinal pathogens. Fluid enrichments, such as Selenite Broth, should be used with Brilliant Green.

Packaging

Brilliant Green Agar	Code No.	7117A	500 g
		7117B	2 kg
		7117C	10 kg

References

1. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. **Kristensen, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren Fur Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville. MD.
6. **Federal Register.** 1993. Chicken disease caused by *Salmonella enteritidis*; proposed rule. Fed. Regist. **58**:41048-41061.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.
8. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars: New media for isolation of enteric pathogens. Am J. Clin. Pathol. **44**:471.

Technical Information

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BRILLIANT GREEN AGAR W/ SULFADIAZINE (7310)

Intended Use

Brilliant Green Agar w/ Sulfadiazine is used for the selective enrichment of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem. Infection with non-typhi *Salmonella* spp. often causes mild, self-limiting illness.¹ 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.

Brilliant Green Agar was first described by Kristensen et al.² and later modified by Kauffman.³ The outstanding selectivity of this medium permits the use of moderately heavy inocula, evenly distributed over the surface. The addition of sulfonamides into Brilliant Green Agar further inhibits *Escherichia coli* and *Proteus* spp. Brilliant Green Agar, abbreviated as BGA, with Sulfadiazine is recommended for the isolation of *Salmonella* from foods, especially eggs.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are the carbon and nitrogen sources used for general growth requirements in BGA w/ Sulfadiazine. Yeast Extract supplies B-complex vitamins, and Lactose and Sucrose are the carbohydrates in this medium. In the presence of Phenol Red, a pH indicator, nonlactose and/or nonsucrose-fermenting *Salmonella* will produce red colonies. Sulfadiazine and Brilliant Green are the selective agents, inhibiting gram-positive organisms and many gram-negative bacteria, except *Salmonella*. Sodium Chloride maintains the osmotic balance. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Lactose	10 g
Sucrose.....	10 g
Brilliant Green	0.0125 g
Phenol Red	0.08 g
Sulfadiazine.....	0.08 g
Agar	20 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 58 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a green tint.

Prepared Appearance: Prepared medium is brown-green and slightly opalescent.

Expected Cultural Response: Cultural response on Brilliant Green Agar w/ Sulfadiazine at 35°C for 18 - 24 hours incubation.

Microorganism	Response	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	----
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	yellow to green colonies
<i>Salmonella choleraeuis</i> ATCC® 13076	good growth	red colonies
<i>Salmonella typhi</i> ATCC® 19430	poor growth	red colonies
<i>Salmonella typhimurium</i> ATCC® 14028	good growth	red colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	----

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for instructions on specific material being tested for *Salmonella*.^{1,4-7}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brilliant Green Agar w/ Sulfadiazine	Code No.	7310A	500 g
		7310B	2 kg
		7310C	10 kg

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
2. **Kristense, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, bromthymol blue, bromcresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **U. S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRILLIANT GREEN BILE BROTH 2% (7119)

Intended Use

Brilliant Green Bile Broth 2% is used for the detection of coliform bacteria in water, food, and dairy products.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative 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 this group, but organisms such as *Aeromonas* spp. may also be included. Procedures to detect and confirm coliforms are used in testing water, foods, dairy products and other materials.¹⁻⁵ Brilliant Green Bile Broth 2% is used to confirm a positive presumptive test result.

Brilliant Green Bile Broth 2% is also referred to as Brilliant Green Bile Broth, Brilliant Green Lactose Broth, Brilliant Green Lactose Bile Broth and Brilliant Green Lactose Bile Broth, 2%.

Principles of the Procedure

Enzymatic Digest of Gelatin is the carbon and nitrogen source used for general growth requirements in Brilliant Green Bile Broth 2%. Oxbile and Brilliant Green inhibit gram-positive bacteria and many gram-negative bacteria, other than coliforms. Lactose is a carbohydrate source. Bacteria that ferment lactose and produce gas are detected.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Lactose	10 g
Oxbile	20 g
Brilliant Green	0.0133 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 40 g of the medium in one liter of purified water until evenly dispersed.
2. Distribute into tubes containing inverted fermentation Durham vials. Autoclave at 121°C for no longer than 15 minutes. To avoid entrapment of bubbles in the fermentation tubes, allow the autoclave to cool at least to 75°C before opening.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green beige.

Prepared Appearance: Prepared medium is emerald green and clear.

Expected Cultural Response: Cultural response in Brilliant Green Bile Broth 2% at 35°C for 18 - 48 hours incubation.

Microorganism	Response	Reactions (Gas)
<i>Enterobacter aerogenes</i> ATCC® 13048	good growth	positive
<i>Enterococcus faecalis</i> ATCC® 29212	marked to complete inhibition	negative
<i>Escherichia coli</i> ATCC® 25922	good growth	positive
<i>Staphylococcus aureus</i> ATCC® 25923	marked to complete inhibition	negative

The organisms listed are the minimum that should be used for quality control testing.

BRILLIANT GREEN AGAR W/ SULFAPYRIDINE (7299)

Intended Use

Brilliant Green Agar w/ Sulfapyridine is used for the selective enrichment of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem. Infection with non-typhi *Salmonella* spp. often causes mild, self-limiting illness.¹ Illness results from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella* spp. Many cases of *Salmonella* related gastroenteritis result from improper handling of poultry products.

Brilliant Green Agar was first described by Kristensen et al.² and later modified by Kauffmann.³ The outstanding selectivity of this medium permits the use of moderately heavy inocula evenly distributed over the surface. The addition of sulfonamides into Brilliant Green Agar further inhibits *Escherichia coli* and *Proteus* spp. Osborne and Stokes used 0.1% Sodium Sulfapyridine to enhance the recovery of *Salmonella* from whole egg and egg yolk.⁴

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are the carbon and nitrogen source used for general growth requirements in this medium. Yeast Extract supplies B-complex vitamins. Lactose and Sucrose are the carbohydrates. In the presence of Phenol Red, a pH indicator, nonlactose and/or nonsucrose-fermenting *Salmonella* spp. will produce red colonies. Sodium Sulfapyridine and Brilliant Green are the selective agents, inhibiting gram-positive organisms and many gram-negative bacteria, except *Salmonella*. Sodium Chloride maintains the osmotic balance. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Lactose	10 g
Sucrose.....	10 g
Brilliant Green	0.0125 g
Phenol Red	0.08 g
Sodium Sulfapyridine	1 g
Agar	20 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 59 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a green tint.

Prepared Appearance: Prepared medium is brown-green, and slightly opalescent, and trace to slightly hazy.

Expected Cultural Response: Cultural response on Brilliant Green Agar w/ Sulfapyridine at 35°C for 18 - 24 hours incubation.

Microorganism	Response	Reaction
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	yellow colonies
<i>Salmonella choleraesuis</i> ATCC® 13076	growth	red colonies
<i>Salmonella typhi</i> ATCC® 19430	partial to complete inhibition	red colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	red colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	----

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for instructions on specific material being tested for *Salmonella*.^{1,5-8}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brilliant Green Agar w/ Sulfapyridine	Code No.	7299A	500 g
		7299B	2 kg
		7299C	10 kg

References

1. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
2. **Kristensen, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, bromthymol blue, bromcresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **Osborne, W. W., and J. L. Stokes.** 1955. The determinations of *Salmonellae* in foods. Ottawa: Food and Drug Laboratories.
5. **U. S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
7. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

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BRILLIANT GREEN AGAR (7117)

Intended Use

Brilliant Green Agar is used for the selective isolation of *Salmonella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *Salmonella typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and/or neurological damage.¹ Infection can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella*. U. S. federal guidelines require various poultry products routinely monitored before distribution for human consumption.

Kristensen, Lester, and Jurgens first described the use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella*.² The report described the medium as useful for the differentiation of "paratyphoid B" from other intestinal gram-negative bacilli.² Kaufmann modified their formula and used Brilliant Green Agar in addition to Tetrathionate Broth for the isolation of *Salmonella* from stool specimens.³

Brilliant Green Agar is recommended for use in testing clinical specimens.^{1,4} The outstanding selectivity of this medium permits use of moderately heavy inocula, which should be evenly distributed over the surface. Brilliant Green Agar is used in the microbial limits test.⁵ Brilliant Green Agar supplemented with novobiocin is used in food testing.⁶

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide sources of nitrogen, amino acids, and carbon. Yeast Extract supplies vitamins required for organism growth. Sodium Chloride maintains the osmotic balance of the medium. Lactose and Sucrose are the carbohydrates in the medium. Brilliant Green inhibits gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Phenol Red is the pH indicator and turns the medium yellow with the formation of acid when lactose and/or sucrose is fermented. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Lactose	10 g
Sucrose.....	10 g
Brilliant Green	0.0125 g
Phenol Red	0.08 g
Agar	20 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 58 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a green tint.

Prepared Appearance: Prepared medium is brown-green, slightly opalescent, and may be trace to slightly hazy.

Expected Cultural Response: Cultural response on Brilliant Green Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	partial inhibition	green colonies
<i>Salmonella choleraesuis</i> ATCC® 13076	growth	pink colonies
<i>Salmonella typhi</i> ATCC® 19430	partial inhibition	pink colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	pink colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For isolation of *Salmonella* from clinical specimens, inoculate fecal specimens and rectal swabs on the first quadrant of Brilliant Green Agar and streak for isolation. Incubate plates at 35°C. Examine plates after 18 – 24 hours for colonies with characteristic morphologies associated with *Salmonella* spp. Refer to appropriate references or standard methods for other applications.

Results

Typical *Salmonella* spp. colonies are opaque and pink. The few lactose and/or sucrose fermenting Organisms that grow are readily differentiated due to formation of green colonies. Brilliant Green Agar is not suitable for the isolation of *S. typhi* or *Shigella* spp., although some strains of *S. typhi* may grow.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Colonies of *Salmonella* spp. can be red, pink, or white depending on length of incubation and strain.⁷
2. Medium is normally orange-brown, however after incubation it can turn bright red and return to normal color at room temperature.⁷
3. Taylor showed that slow lactose fermenters, *Proteus*, *Citrobacter*, and *Pseudomonas* may grow on Brilliant Green Agar as red colonies.⁸
4. Other primary plating medium such as MacConkey Agar should be used when testing for intestinal pathogens. Fluid enrichments, such as Selenite Broth, should be used with Brilliant Green.

Packaging

Brilliant Green Agar	Code No.	7117A	500 g
		7117B	2 kg
		7117C	10 kg

References

1. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. **Kristensen, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren Fur Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
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7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.
8. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars: New media for isolation of enteric pathogens. Am J. Clin. Pathol. **44**:471.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

Test Procedure

Refer to appropriate references for specific instructions for the material being tested. ¹⁻⁵

1. Subculture from a presumptive positive coliform specimen in Lauryl Sulfate Broth or from typical coliform colonies on Violet Red Bile Agar to tubes of Brilliant Green Bile Broth 2%.
2. Incubate at 35°C for 48 ± 2 hours.
3. Examine for bubbles (gas) in the fermentation tube.

Results

Positive: Bubbles (gas) in fermentation tube.

Negative: No bubbles (gas) in fermentation tube.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brilliant Green Bile Broth 2% Code No.	7119A	500 g
	7119B	2 kg
	7119C	10 kg

References

1. **U. S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

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BRILLIANT GREEN BILE BROTH 2% w/ MUG (7344)

Intended Use

Brilliant Green Bile Broth 2% w/ MUG is used for the detection of coliforms and the fluorogenic detection of *Escherichia coli*.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative anaerobic, gram-negative, non-sporeforming, bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. *Escherichia coli* is a member of the fecal coliform group. The presence of *E. coli* is indicative of fecal contamination.¹

Procedures to detect and confirm the presence of coliforms are used in testing water, foods, dairy products, and other materials.¹⁻⁵ The procedures begin with a presumptive test, and when positive, confirmed using Brilliant Green Bile Broth 2%. The addition of the fluorogenic compound, MUG (4-Methylumbelliferyl-®-D-glucuronide) to this medium permits the rapid detection of *E. coli* when medium is observed for fluorescence using a long-wave UV light source.

Principles of the Procedure

Enzymatic Digest of Gelatin provides the nitrogen and vitamin sources in Brilliant Green Bile Broth 2% w/ MUG. Lactose is the carbohydrate energy source. Ox bile and Brilliant Green inhibit gram-positive bacteria and many gram-negative bacteria, other than coliforms. The substrate MUG (4-Methylumbelliferyl-®-D-glucuronide) produces a blue fluorescence when hydrolyzed by the enzyme ®-glucuronidase, produced by most *E. coli*.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Lactose	10 g
Ox bile	20 g
MUG (4-Methylumbelliferyl®-D-glucuronide).....	0.05 g
Brilliant Green	0.0133 g
Final pH: 7.2 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system and skin.

Directions

1. Dissolve 40 g of the medium in one liter of purified water.
2. Dispense into tubes containing inverted fermentation tubes.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and greenish-beige.

Prepared Appearance: Prepared medium is emerald green and clear.

Expected Cultural Response: Cultural response in Brilliant Green Bile Broth 2% w/ MUG at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions	
		Gas	Fluorescence
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	positive	negative
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	negative	negative
<i>Escherichia coli</i> ATCC® 25922	growth	positive	positive
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	negative	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Follow the methods and procedures for the test being performed using Brilliant Green Bile Broth 2% w/ MUG.

Results

Observe inoculated tube for characteristic growth, gas production, and fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence throughout the tube when exposed to long wave UV light. Non-*E.coli* coliforms may produce gas but do not fluoresce.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Glucuronidase-negative strains of *E. coli* have been encountered⁶⁻⁹ and will be missed by this procedure.
3. Strains of *Salmonella* spp. and *Shigella* spp. that produce glucuronidase may infrequently be encountered.¹⁰ 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.

Packaging

Brilliant Green Bile Broth 2% w/ MUG	Code No.	7344A	500 g
		7344B	2 kg
		7344C	10 kg

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1993. Coliform and other indicator bacteria, p. 247-269. In R. T. Marshall (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington D.C.
4. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler.** 1995. *Escherichia coli* and the coliform bacteria, p. 4.01-4.29. In Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Andrews, W. H.** 1995. Microbial methods, p. 1-119. In Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
6. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of β -D-Glucuronidase-negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.
7. **Hansen, W., and E. Yourassowsky.** 1984. Detection of β -D-Glucuronidase in lactose fermenting members of the family enterobacteriaceae and its presence in bacterial urine cultures. J. Clin. Microbiol. **20**:1177-1179.
8. **Kilian, M. and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. Acta. Pathol. Microbiol. Scand. Sect. B **84**:245-251.
9. **Mates, A., and M. Shaffer.** 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriol. **67**:343-346.
10. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. J. Food Sc. **50**:1736-1746.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRILLIANT GREEN BILE BROTH 2% (7119)

Intended Use

Brilliant Green Bile Broth 2% is used for the detection of coliform bacteria in water, food, and dairy products.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative 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 this group, but organisms such as *Aeromonas* spp. may also be included. Procedures to detect and confirm coliforms are used in testing water, foods, dairy products and other materials.¹⁻⁵ Brilliant Green Bile Broth 2% is used to confirm a positive presumptive test result.

Brilliant Green Bile Broth 2% is also referred to as Brilliant Green Bile Broth, Brilliant Green Lactose Broth, Brilliant Green Lactose Bile Broth and Brilliant Green Lactose Bile Broth, 2%.

Principles of the Procedure

Enzymatic Digest of Gelatin is the carbon and nitrogen source used for general growth requirements in Brilliant Green Bile Broth 2%. Oxbile and Brilliant Green inhibit gram-positive bacteria and many gram-negative bacteria, other than coliforms. Lactose is a carbohydrate source. Bacteria that ferment lactose and produce gas are detected.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Lactose	10 g
Oxbile	20 g
Brilliant Green	0.0133 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 40 g of the medium in one liter of purified water until evenly dispersed.
2. Distribute into tubes containing inverted fermentation Durham vials. Autoclave at 121°C for no longer than 15 minutes. To avoid entrapment of bubbles in the fermentation tubes, allow the autoclave to cool at least to 75°C before opening.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green beige.

Prepared Appearance: Prepared medium is emerald green and clear.

Expected Cultural Response: Cultural response in Brilliant Green Bile Broth 2% at 35°C for 18 - 48 hours incubation.

Microorganism	Response	Reactions (Gas)
<i>Enterobacter aerogenes</i> ATCC® 13048	good growth	positive
<i>Enterococcus faecalis</i> ATCC® 29212	marked to complete inhibition	negative
<i>Escherichia coli</i> ATCC® 25922	good growth	positive
<i>Staphylococcus aureus</i> ATCC® 25923	marked to complete inhibition	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific instructions for the material being tested. ¹⁻⁵

1. Subculture from a presumptive positive coliform specimen in Lauryl Sulfate Broth or from typical coliform colonies on Violet Red Bile Agar to tubes of Brilliant Green Bile Broth 2%.
2. Incubate at 35°C for 48 ± 2 hours.
3. Examine for bubbles (gas) in the fermentation tube.

Results

Positive: Bubbles (gas) in fermentation tube.

Negative: No bubbles (gas) in fermentation tube.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brilliant Green Bile Broth 2% Code No.	7119A	500 g
	7119B	2 kg
	7119C	10 kg

References

1. **U. S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

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BRUCELLA AGAR (7120)

Intended Use

Brucella Agar is used for the cultivation of *Brucella* spp. and other fastidious microorganisms.

Product Summary and Explanation

Brucella Agar is prepared according to the APHA formula for Albimi Broth.¹ Brucella Agar is a general purpose medium for the cultivation of *Brucella* spp. and fastidious microorganisms including *Streptococcus pneumoniae*, *Streptococcus viridans*, and *Neisseria meningitidis*.² With the addition of blood, Brucella Agar is used to determine bacterial hemolytic reactions.² Brucella Agar can be used as a base for the isolation of *Campylobacter* spp.²

Brucellosis is a zoonotic disease with a domestic-animal reservoir.³ Transmission by milk, milk products, meat, and direct contact with infected animals are the usual routes of exposure.³

Principles of the Procedure

The nitrogen and carbon source is provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue in Brucella Agar. Yeast Extract is the vitamin source. Dextrose is the carbohydrate. Sodium Chloride maintains the osmotic environment. Sodium Bisulfite is added to enhance growth. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Yeast Extract.....	2 g
Sodium Chloride	5 g
Dextrose.....	1 g
Sodium Bisulfite	0.1 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. *Brucella* spp. are classified as Biosafety Level 3 pathogens. Procedures with live cultures and antigens must be confined to Class II biological safety cabinet (BSC).³
3. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 43 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and yellow beige.

Expected Cultural Response: Cultural response on Brucella Agar at 35°C under 3% CO₂ after 18 - 96 hours incubation.

Microorganism	Response
<i>Brucella abortus</i> ATCC® 4315	growth
<i>Brucella melitensis</i> ATCC® 4309	growth
<i>Brucella suis</i> ATCC® 4314	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the isolation and identification of *Brucella* spp.^{4,5}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brucella Agar	Code No.	7120A	500 g
		7120B	2 kg
		7120C	10 kg

References

1. **Hausler, W. J. (ed.).** 1976. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 110-114. Williams & Wilkins, Baltimore, MD.
3. **Moyer, N. P., and L. A. Holcomb.** 1995. *Brucella*, p. 549-555. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BRUCELLA BROTH (7121)

Intended Use

Brucella Broth is used for the cultivation of *Brucella* spp. and other fastidious microorganisms.

Product Summary and Explanation

Brucella Broth is prepared according to the APHA formula for Albimi Broth.¹ Brucella Broth is a general purpose medium for the cultivation of *Brucella* spp. and fastidious microorganisms including, *Streptococcus pneumoniae*, *Streptococcus viridans*, and *Neisseria meningitidis*.² *Brucella* spp. is the causative agent for brucellosis, a zoonotic disease with a domestic-animal reservoir.³ Transmission by milk, milk products, meat, and direct contact with infected animals is the usual route of exposure.³

Brucella Broth is recommended for the isolation of *Brucella* spp. from blood cultures,^{4,5} and specified in standard methods for the examination of food.⁶

Principles of the Procedure

The nitrogen and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue in Brucella Broth. Yeast Extract is the vitamin source in this medium. Dextrose is the carbohydrate source, and Sodium Chloride maintains the osmotic environment. Sodium Bisulfite is added to enhance growth.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Yeast Extract.....	2 g
Sodium Chloride	5 g
Dextrose.....	1 g
Sodium Bisulfite	0.1 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. *Brucella* spp. are classified as Biosafety Level 3 pathogens. Procedures with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).³
3. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 28 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear and light amber.

Expected Cultural Response: Cultural response in Brucella Broth at 35°C under 3% CO₂ after 18 - 72 hours incubation.

Microorganism	Response
<i>Brucella abortus</i> ATCC® 4315	growth
<i>Brucella melitensis</i> ATCC® 4309	growth
<i>Brucella suis</i> ATCC® 4314	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the isolation and identification of *Brucella* spp.^{4,5}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Brucella Broth	Code No.	7121A	500 g
		7121B	2 kg
		7121C	10 kg

References

1. **Hausler, W. J. (ed.).** 1976. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 110-114. Williams & Wilkins, Baltimore, MD.
3. **Moyer, N. P., and L. A. Holcomb.** 1995. *Brucella*, p. 549-555. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BUFFERED LISTERIA ENRICHMENT BROTH (7579)

Intended Use

Buffered Listeria Enrichment Broth is used for selective enrichment of *Listeria* spp.

Product Summary and Explanation

Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann,¹ is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.² Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey, frankfurters, coleslaw, pasteurized milk, Mexican style cheese, and pate'.⁴ *Listeria* spp. are ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, and river water.⁵

Buffered Listeria Enrichment Broth, a modification of the formula by Lovett et al.,⁶ was developed after subsequent work concluded that enrichment properties can be improved by increasing the buffering capacity of the medium with the addition of Disodium Phosphate. *Listeria* spp. grow over a pH range of 5.0 - 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, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Soybean Meal, and Yeast Extract provides nitrogen, vitamins, and minerals in Buffered Listeria Enrichment Broth. Dextrose is the carbohydrate source. Sodium Chloride maintains osmotic balance of the medium. Monopotassium Phosphate, Dipotassium Phosphate, and Disodium Phosphate are the buffering agents. Nalidixic Acid inhibits growth of gram-negative organisms. Acriflavin inhibits gram-positive bacteria. Cyclohexamide is used to inhibit growth of saprophytic fungi.

Formula / Liter

Enzymatic Digest of Casein	17 g
Enzymatic Digest of Soybean Meal	3 g
Yeast Extract.....	6 g
Dextrose.....	2.5 g
Sodium Chloride	5 g
Monopotassium Phosphate	1.35 g
Dipotassium Phosphate.....	2.5 g
Disodium Phosphate.....	9.6 g
Cycloheximide.....	0.05 g
Nalidixic Acid.....	0.04 g
Acriflavin	0.015 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. TOXIC. Harmful by inhalation and if swallowed. Possible risk to unborn child.

Directions

1. Dissolve 47 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and yellow to tan.

Prepared Appearance: Prepared medium is yellow with a green tint.

Expected Cultural Response: Cultural response in Buffered Listeria Enrichment Broth at 30°C after 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	good growth
<i>Staphylococcus aureus</i> ATCC® 25923	suppressed at 18 – 24 hours

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Use recommended laboratory procedures for isolating *Listeria* in food samples.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Buffered Listeria Enrichment Broth	Code No.	7579A	500 g
		7579B	2 kg
		7579C	10 kg

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bact. **29**:407-439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
4. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
5. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
6. **Lovette, J., D. W. Frances, and J. M. Hunt.** 1987. *Listeria monocytogenes* In raw milk: detection, incidence and pathogenicity. J. Food Prot. **50**:188-192.
7. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BUFFERED PEPTONE WATER (BROTH) (7418)

Intended Use

Buffered Peptone Water is used for the non-selective pre-enrichment of *Salmonella* spp. from food.

Product Summary and Explanation

Edel and Kamelmacher¹ found that food preservation techniques involving heat, desiccation, preservatives, high osmotic pressure, or pH changes cause sublethal injury to *Salmonella* spp. Pre-enrichment in a non-selective medium allows for repair of cell damage and facilitates the recovery of *Salmonella*. 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 pre-enrichment period and allows 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 is used in standard methods.⁴

Principles of the Procedure

Peptone is the nitrogen, carbon, vitamin, and mineral sources in Buffered Peptone Water. Sodium Chloride maintains the osmotic balance. Phosphates buffer the medium.

Formula / Liter

Peptone..... 10 g
Sodium Chloride 5 g
Disodium Phosphate..... 3.5 g
Monopotassium Phosphate 1.5 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 20 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium, if necessary.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear and pale yellow.

Expected Cultural Response: Cultural response in Buffered Peptone Water at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	good growth
<i>Salmonella typhimurium</i> ATCC® 14028	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Buffered Peptone Water.

Results

Growth is indicated by turbidity.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Buffered Peptone Water	Code No.	7418A	500 g
		7418B	2 kg
		7418C	10 kg

References

1. **Edel, W., and E. H. Kampelmacher.** 1973. Bull World Hlth. Org. **48**:167-174.
2. **Angelotti, R.** 1963. Microbiological quality of foods. Academic Press, New York.
3. **Sadovski, A. Y.** 1977. J. Food Technol. **12**:85-91.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CAMPY BLOOD FREE SELECTIVE MEDIUM (CCDA) (7527)

Intended Use

Campy Blood Free Selective Medium (CCDA) is used with cefoperazone for the selective isolation of *Campylobacter* spp.

Product Summary and Explanation

Many experts consider *Campylobacter* to be the leading cause of enteric illness in the US.¹ *Campylobacter* spp. can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea.¹ These pathogens are highly infective, and transmitted by contaminated food or water.

Campy Blood Free Selective Medium (CCDA) was described by Bolton et al.² This medium was formulated to replace blood with charcoal, ferrous sulfate, and sodium pyruvate. To improve selectivity, cefoperazone replaced cephazolin in the original formulation.³ Bolton et al.⁴ recommended incubating inoculated plates at 37°C to improve isolation rates. Yeast and fungal contaminants are inhibited with the addition of amphotericin B.

Campy Blood Free Selective Medium (CCDA) is recommended for food testing.^{1,5}

Principles of the Procedure

Nutrient Broth No. 2 and Casein Acid Hydrolysate provides nitrogen and vitamin sources in this medium. Charcoal absorbs toxic compounds and metabolites. Sodium Desoxycholate and Cefoperazone are selective agents to inhibit enteric flora. Ferrous Sulfate and Sodium Pyruvate are present as oxygen scavengers. Agar is the solidifying agent.

Formula / Liter

Nutrient Broth No. 2	25 g
Charcoal.....	4 g
Casein Acid Hydrolysate	3 g
Sodium Desoxycholate	1 g
Ferrous Sulfate	0.25 g
Sodium Pyruvate.....	0.25 g
Agar	12 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Cefoperazone, 32 mg

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful by inhalation, ingestion, and through skin absorption.

Directions

1. Suspend 45.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and aseptically add 4 mL of a filtered sterilized aqueous solution containing 32 mg of cefoperazone.
5. Mix well and pour into petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and gray-black.

Prepared Appearance: Prepared medium is gray-black.

Expected Cultural Response: Cultural response on Campy Blood Free Selective Medium (CCDA) at 37°C after 18 - 48 hours in an atmosphere consisting of approximately 5 - 6% oxygen, 3 - 10% carbon dioxide, and 84 - 85% nitrogen.

Microorganism	Response
<i>Campylobacter jejuni</i> ATCC® 33291	good growth
<i>Escherichia coli</i> ATCC® 25922	Inhibited

The organisms listed are the minimum that should be used for quality control testing.

Note: Quality Control Laboratory sample was tested with the addition of cefoperazone.

Test Procedure

1. Inoculate the specimen directly onto the surface of the prepared Campy Blood Free Selective Medium (CCDA). If an enrichment broth is required, refer to the appropriate references.^{1,5,6}
2. Streak for isolation.
3. Incubate inoculated plates at 37°C or 42°C in an atmosphere composed of 5 - 6% oxygen, 3 - 10% carbon dioxide and 84 - 85% nitrogen for 24 - 48 hours. Selective temperatures are required for certain *Campylobacter* spp. Refer to appropriate references on the proper temperature for the targeted *Campylobacter* spp.¹

Results¹

Campylobacter colonies are round to irregular with smooth edges. They may have translucent, white colonies to spreading, flat, transparent growth. Some strains appear tan or slightly pink. Normal enteric flora is completely to markedly inhibited.

Typically, *Campylobacter* spp. are oxidase positive and catalase positive. For complete identification of species and biotype, refer to the appropriate procedures for biochemical reactions.^{1,4}

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Campy Blood Free Selective Medium (CCDA)	Code No.	7527A	500 g
		7527B	2 kg
		7527C	10 kg

References

1. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Bolton, F. J., D. N. Hutchinson, and D. Coates.** 1984. J. Clin. Microbiol. **19**:169-171.
3. **Bolton, F. J., and D. N. Hutchinson.** 1984. J. Clin. Pathol. **34**:956-957.
4. **Bolton, F. J., D. N. Hutchinson, and G. Parker.** 1988. Eur. J. Clin. Microbiol. Infect Dis. **7**:155-160.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CAMPY SELECTIVE AGAR BASE (PRESTON) (7443)

Intended Use

Campy Selective Agar Base (Preston) is used with antimicrobics for the selective isolation of *Campylobacter jejuni* and *Campylobacter coli*.

Product Summary and Explanation

Many experts consider *Campylobacter* to be the leading cause of enteric illness in the US.¹ *Campylobacter* spp. can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea.¹ These pathogens are highly infective, and transmitted by contaminated food or water.

Campy Selective Agar Base (Preston) is based on the formulation described by Bolton and Robertson.² This formula, with the addition of the Preston Supplement, was developed to isolate *Campylobacter* spp. from human, animal, and environmental specimens. The Preston formulation demonstrated improved recovery and selectivity of *Campylobacter* spp. in comparative studies with other selective media (Skirrow, Butzler, Blaser and Campy-Blood Agar).³

Principles of the Procedure

Enzymatic Digest of Animal Tissue and Enzymatic Digest of Casein are the nitrogen and vitamin source in this medium. Sodium Chloride provides the osmotic environment, Agar is the solidifying agent. Antimicrobics are added to suppress normal enteric flora, and enhance the growth of *Campylobacter* spp. The addition of 5% lysed horse blood provides essential growth factors.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	10 g
Enzymatic Digest of Casein	10 g
Sodium Chloride	5 g
Agar	12 g

Final pH: 7.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobials / 10 mL

Polymyxin B	5000 IU
Trimethoprim	10 mg
Rifampin	10 mg
Cycloheximide	100 mg

Precautions

1. For In Vitro Diagnostic Use.
2. Follow standard laboratory policies when handling and disposing of contaminated material.
3. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 37 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and aseptically add 5% lysed horse blood and 10 mL of a filtered sterilized aqueous solution containing 5000 IU polymyxin B, 10 mg trimethoprim, 10 mg rifampin, and 100 mg cycloheximide.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium with 5% lysed horse blood is red, clear to trace hazy.

Expected Cultural Response: Cultural response at 37°C after 18 - 48 hours incubation on 5% horse blood plates in an atmosphere consisting of approximately 5 - 6% oxygen, 3 - 10% carbon dioxide and 84 - 85% nitrogen.

Microorganism	Response
<i>Campylobacter jejuni</i> ATCC® 29428	growth
<i>Campylobacter jejuni</i> ATCC® 33291	growth
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited
<i>Proteus mirabilis</i> ATCC® 12453	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate the specimen directly onto the surface of the prepared Campy Selective Agar Base (Preston).
2. Streak for isolation.
3. Incubate inoculated plates at 37°C or 42°C in an atmosphere composed of 5 - 6% oxygen, 3 - 10% carbon dioxide and 84 - 85% nitrogen for 24 - 48 hours. Selective temperatures are required for certain strains of *Campylobacter* spp. Refer to appropriate references on the proper temperature and microaerophilic environment of *Campylobacter* spp.¹

Results¹

Campylobacter colonies are round to irregular with smooth edges. They may have translucent, white colonies to spreading, flat, transparent growth. Some strains appear tan or slightly pink. Normal enteric flora is completely to markedly inhibited.

Typically, *Campylobacter* spp. are oxidase positive and catalase positive. For complete identification of species and biotype, refer to the appropriate procedures for biochemical reactions.^{1,4}

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Campy Selective Agar Base (Preston)	Code No.	7443A	500 g
		7443B	2 kg
		7443C	10 kg

References

1. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Bolton, F. J., and L. Robertson.** 1982. J. Clin. Microbiol. **35**:462-467.
3. **Bolton, F. J., D. Coates, P. M. Hinchliffe, and L. Robertson.** 1983. J. Clin. Pathol. **36**:78-83.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CAMPYLOBACTER ENRICHMENT BROTH (7526)

Intended Use

Campylobacter Enrichment Broth is used with antimicrobics for the selective enrichment of *Campylobacter* spp.

Product Summary and Explanation

Many experts consider *Campylobacter* to be the leading cause of enteric illness in the US.¹ *Campylobacter* spp. can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea.¹ These pathogens are highly infective, and transmitted by contaminated food or water.

Campylobacter spp. are microaerophilic, very small, curved, thin, Gram-negative rods.¹ Microaerophilic organisms have a tendency to be more sensitive to toxic forms of oxygen.² Campylobacter Enrichment Broth, along with nutritional ingredients, contains compounds which enhance the aerotolerance of microaerophilic bacteria by suppressing the toxic form of oxygen.² Campylobacter Enrichment Broth is recommended in food testing.¹

Principles of the Procedure

Enzymatic Digest of Animal Tissue, Lactalbumin Hydrolysate, and Yeast Extract provide nitrogen, carbon, amino acids, and vitamins in Campylobacter Enrichment Broth. Hemin and Lysed Horse Blood provide essential growth factors. Sodium Chloride maintains the osmotic balance of the medium. Sodium Pyruvate, Sodium Metabisulphite, and Sodium Carbonate increase the aerotolerance of *Campylobacter* spp. by acting as oxygen scavengers. The addition of cefoperazone, cycloheximide, trimethoprim, and vancomycin are selective agents for heavily contaminated samples.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	10 g
Lactalbumin Hydrolysate.....	5 g
Yeast Extract.....	5 g
Sodium Chloride	5 g
Hemin.....	0.01 g
Sodium Pyruvate.....	0.5 g
α -Ketoglutamic Acid.....	1 g
Sodium Metabisulphite.....	0.5 g
Sodium Carbonate	0.6 g

Final pH: 7.4 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobial / 10 mL of Ethanol

Cefoperazone	20 mg
Cycloheximide	50 mg
Trimethoprim	20 mg
Vancomycin	20 mg

Enrichment

Lysed Horse Blood	50 mL

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful if swallowed, inhaled, ingested, or absorbed through skin.

Directions

1. Dissolve 27.6 g of the medium in one liter of purified water.
2. Allow powder to soak for 10 minutes.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and aseptically add 50 mL of lysed horse blood and 10 mL of ethanol containing 20 mg of cefoperazone, 50 mg of cycloheximide, 20 mg trimethoprim, and 20 mg vancomycin.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and amber.

Expected Cultural Response: Cultural response, after incubation in *Campylobacter* Enrichment Broth, on Blood Agar Base No. 2 after 24 - 48 hour incubation at 35°C.

Microorganism	Response	
	w/ Antibiotic	w/o Antibiotic
<i>Campylobacter jejuni</i> ATCC® 29428	good growth	good growth
<i>Campylobacter jejuni</i> ATCC® 33291	good growth	good growth
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	good growth
<i>Escherichia coli</i> ATCC® 25922	inhibited	good growth
<i>Proteus mirabilis</i> ATCC® 12453	inhibited	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to the appropriate procedure for the material being testing on the isolation of *Campylobacter* spp. Refer to standard methods for food testing.¹

Results¹

Campylobacter colonies are round to irregular with smooth edges. They may have translucent, white colonies to spreading, flat, transparent growth. Some strains appear tan or slightly pink. Normal enteric flora is completely to markedly inhibited. Typically, *Campylobacter* spp. are oxidase positive and catalase positive. For complete identification of species and biotype, refer to the appropriate procedures for biochemical reactions.^{1,3}

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Campylobacter Enrichment Broth	Code No.	7526A	500 g
		7526B	2 kg
		7526C	10 kg

References

1. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed., AOAC International, Gaithersburg, MD.
2. **George, H. A., P. S. Hoffman, and N. R. Krieg.** 1978. J. Clin. Micro. 8:36-41.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CARY and BLAIR TRANSPORT MEDIUM (7253)

Intended Use

Cary and Blair Transport Medium is used for the collection, transport, and preservation of microorganisms.

Product Summary and Explanation

Transport media are chemically defined, semisolid, non-nutritive, phosphate buffered media that provide a reduced environment. Transport media are developed to maintain the viability of microorganisms without significant increase in growth. In 1964, Cary and Blair modified a transport medium, Stuart's Medium, by substituting inorganic phosphates for glycerophosphate and raising the pH to 8.4¹ This modified medium was effective in maintaining the viability of *Salmonella* and *Shigella* in fecal samples.^{2,3} Due to the high pH, Cary and Blair Transport Medium is also effective in maintaining the viability of *Vibrio* for up to four weeks.⁴ Cary and Blair Transport Medium is currently recommended for fecal and rectal samples.

Principles of the Procedure

In Cary and Blair Transport Medium, Sodium Thioglycollate suppresses oxidative changes and provides a reduced environment. Disodium Phosphate is a buffering agent. Sodium Chloride and Calcium Chloride provides essential ions that help maintain osmotic balance while controlling permeability of bacterial cells. Agar is the solidifying agent.

Formula / Liter

Sodium Thioglycollate..... 1.5 g
Disodium Phosphate..... 1.1 g
Sodium Chloride 5 g
Calcium Chloride..... 0.09 g
Agar 5 g

Final pH: 8.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 12.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense the medium into 6 – 8 mL capacity screw cap vials to within 5 mm of the top.
4. Tighten caps and steam for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and white.

Prepared Appearance: Prepared medium is white and hazy.

Expected Cultural Response: Viability is maintained for at least 24 hours for the following microorganisms.

Microorganism	Response
<i>Haemophilus influenzae</i> ATCC® 35056	good
<i>Neisseria meningitidis</i> ATCC® 13090	good
<i>Staphylococcus aureus</i> ATCC® 25923	good
<i>Streptococcus pyogenes</i> ATCC® 19615	good

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Insert specimen swab(s) into the upper third of the medium in the transport container.
2. Cut or break off the protruding portion of the swab. Tightly screw the lid on vial.
3. Label the vial and submit to the laboratory as soon as possible. Specimens may be refrigerated until ready for shipment.

Results

Survival of bacteria in transport media depends on many factors including type and concentration of bacteria in the specimen, transport medium formulation, temperature and duration of transport, and inoculation to appropriate culture media within 24 hours.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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.
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 coliforms.
3. The condition of the specimen received by the laboratory for culture is a significant variable in recovery and final identification of the pathogen. An unsatisfactory specimen (overgrown by contaminants, containing non-viable organisms, or having the number of pathogens greatly diminished) can lead to erroneous or inconclusive results.

Packaging

Cary and Blair Transport Medium	Code No.	7253A	500 g
		7253B	2 kg
		7253C	10 kg

References

1. **Cary, S. G., and E. B. Blair.** 1964. New transport medium for shipment of clinical specimens. *J. Bacteriol.* **88**:96-98.
2. **Cary, S. G., M. S. Matthew, M. H. Fusillo, and C. Harkins.** 1965. Survival of *Shigella* and *Salmonella* in a new transport medium. *Am. J. Clin. Pathol.* **43**:294-296.
3. **Neuman, D. A., M. W. Benenson, E. Hubster, and Thi Nhu Tuan.** 1971. *N. Am. J. Clin. Path.* **57**:33-34.
4. **Kelly, M. T., F. W. Hickman-Brenner, and J. J. Farmer III.** 1991. *Vibrio*, p. 384-395. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (eds.). *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CARY & BLAIR TRANSPORT MEDIUM, MODIFIED w/ PHENOL RED (7308)

Intended Use

Cary & Blair Transport Medium, Modified w/ Phenol Red is used for the collection, transport, and preservation of microorganisms.

Product Summary and Explanation

Transport media are chemically defined, semisolid, non-nutritive, buffered media that provide a reduced environment. Transport media are developed to maintain the viability of microorganisms without significant increase in growth. In 1964, Cary and Blair modified a transport medium, Stuart's Medium, by substituting inorganic phosphates for glycerophosphate and raising the pH to 8.4.¹ This modified medium was effective in maintaining the viability of *Salmonella* and *Shigella* in fecal samples.^{2,3} Due to the high pH, Cary and Blair Transport Medium is also effective in maintaining the viability of *Vibrio* for up to four weeks.⁴ Cary and Blair Transport Medium is currently recommended for fecal and rectal samples.

Cary & Blair Transport Medium, Modified w/ Phenol Red is similar to the formulation of Cary & Blair Transport Medium, except calcium chloride is omitted and phenol red added.

Principles of the Procedure

Sodium Thioglycollate suppresses oxidative changes, providing a reduced environment in this transport medium. Disodium Phosphate is a buffering agent. Sodium Chloride provides essential ions to maintain osmotic balance while controlling permeability of bacterial cells. Phenol Red detects pH changes, indicating metabolic changes in the specimen. Agar is the solidifying agent.

Formula / Liter

Sodium Thioglycollate 1.5 g
Disodium Phosphate..... 1.1 g
Sodium Chloride 5 g
Phenol Red 0.003 g
Agar 1.6 g

Final pH: 8.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 9.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense the hot medium into 6 – 8 mL capacity screw cap vials to within 0.5 mm of the top.
4. Tighten caps and autoclave vials at 121°C for 15 minutes. Retighten caps if needed.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and white.

Prepared Appearance: Prepared medium is pink, clear to trace hazy.

Expected Cultural Response: Viability is maintained for at least 24 hours for the following microorganisms.

Microorganism	Response
<i>Campylobacter jejuni</i> ATCC® 33291	Recovered an appropriate non-selective medium
<i>Shigella flexneri</i> ATCC® 12022	Recovered an appropriate non-selective medium
<i>Yersinia enterocolitica</i> ATCC® 27729	Recovered an appropriate non-selective medium

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Insert specimen swab(s) into upper third of the medium in transport container.
2. Cut or break off the protruding portion of the swab. Tightly screw the lid on vial.
3. Label the vial and submit to the laboratory as soon as possible. Specimens may be refrigerated until ready for shipment.

Results

Survival of bacteria in transport media depends on many factors including type and concentration of bacteria in the specimen, transport medium formulation, temperature and duration of transport, and inoculation to appropriate culture media within 24 hours.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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.
2. Viability of cells will diminish over time and multiplication or growth of contaminants can occur during prolonged periods of transit. This is particularly true of fecal specimens that contain substantial numbers of coliforms.
3. The condition of the specimen received by the laboratory for culture is a significant variable in recovery and final identification of the pathogen. An unsatisfactory specimen (overgrown by contaminants, containing non-viable organisms, or having the number of pathogens greatly diminished) can lead to erroneous or inconclusive results.

Packaging

Cary & Blair Transport Medium, Modified w/ Phenol Red	Code No. 7308A	500 g
	7308B	2 kg
	7308C	10 kg

References

1. **Cary, S. G., and E. B. Blair.** 1964. New transport medium for shipment of clinical specimens. *J. Bacteriol.* **88**:96-98.
2. **Cary, S. G., M. S. Matthew, M. H. Fusillo, and C. Harkins.** 1965. Survival of *Shigella* and *Salmonella* in a new transport medium. *Am. J. Clin. Pathol.* **43**:294-296.
3. **Neuman, D. A., M. W. Benson, E. Hubster, and Thi Nhu Tuan.** 1971. *N. Am. J. Clin. Path.* **57**:33-34.
4. **Kelly, M. T., F. W. Hickman-Brenner, and J. J. Farmer III.** 1991. *Vibrio*, p. 384-395. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (eds.). *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CASEIN, ACID HYDROLYSATE (7229)

Intended Use

Casein, Acid Hydrolysate is a hydrochloric acid hydrolysate of casein for use in preparing microbiological culture media.

Product Summary and Explanation

Casein is a rich source of amino nitrogen. Treatment with hydrochloric acid hydrolyzes the protein into primarily free amino acids with some short peptide fragments. Tryptophan and vitamins present in the casein are destroyed by the acid hydrolysis. The salt content of this peptone is very high at approximately 36%. While this peptone is used in a number of microbiological media formulations, its main usage is in Mueller Hinton Agar.

Principles of the Procedure

Casein, Acid Hydrolysate is an acid hydrolysate of casein. Casein, Acid Hydrolysate is an excellent source of free amino acids and short peptide fragments, which are required by microorganisms for growth.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and light beige.

Prepared Appearance (2% wt/vol): Prepared medium is clear to brilliant, gold with no or a light precipitate.

pH (2% Solution at 25°C): 6.5 - 7.3

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35°C after 18-24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	poor to fair growth

Test Procedure

Refer to appropriate references for specific procedures using Casein, Acid Hydrolysate.

Results

Refer to appropriate references for test results.

Storage

Store sealed container containing Casein, Acid Hydrolysate at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Casein, Acid Hydrolysate should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Casein, Acid Hydrolysate in its intact container when stored as directed.

Packaging

Casein, Acid Hydrolysate	Code No.	7229A	500 g
		7229B	2 kg
		7229C	10 kg

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CASMAN MEDIUM BASE (7123)

Intended Use

Casman Medium Base is used with blood for the isolation of *Haemophilus influenzae*, *Neisseria gonorrhoeae* and other fastidious microorganisms.

Product Summary and Explanation

In 1947, Casman described an infusion-free medium enriched with 5% blood for fastidious microorganisms incubated anaerobically.¹⁻³ This medium replaced formulas containing fresh meat infusion and heated blood.¹ Casman adjusted the formula after experiments revealed nicotinamide disrupted the action of a blood enzyme that inactivates V factor (NAD).² The concentration of nicotinamide was lowered to support growth of *Neisseria* spp.^{2,3}

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, Yeast Enriched Peptone, and Beef Extract. Niacinamide enhances growth of *N. gonorrhoeae* and *H. influenzae* by impeding the removal of coenzyme (V factor) by nucleotidase from enriched blood. A small amount of Dextrose is added to enhance growth of pathogenic cocci, and p-Aminobenzoic Acid is a B vitamin preservative. Corn Starch is added to ensure any toxic metabolites produced are absorbed, to neutralize glucose inhibition of beta-hemolysis, and enhance growth of *Neisseria* spp.⁴ Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Yeast Enriched Peptone	10 g
Beef Extract	3 g
Niacinamide	0.05 g
p-Aminobenzoic Acid	0.05 g
Dextrose.....	0.5 g
Corn Starch.....	1 g
Sodium Chloride	5 g
Agar	13.5 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 43 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. After cooling medium to 50°C, aseptically add 5% sterile blood and 0.15% sterile water-lysed blood solution (one part blood to three parts water).

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is trace to slightly hazy and light to medium amber. Prepared medium supplemented with 5% sheep blood is opaque and red.

Expected Cultural Response: Cultural response on Casman Medium Base supplemented with blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Haemophilus influenzae</i> ATCC® 35056	growth
<i>Neisseria gonorrhoeae</i> ATCC® 43070	growth
<i>Neisseria lactamica</i> ATCC® 23970	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Neisseria sicca</i> ATCC® 9913	growth
<i>Streptococcus agalactiae</i> ATCC® 13813	growth
<i>Streptococcus pneumoniae</i> ATCC® 6303	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and identification of fastidious microorganisms.

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Niacinamide in concentrations greater than 0.005% may inhibit growth of some *N. gonorrhoeae* strains; however, only slight stimulation of growth of *H. influenzae* occurs with this amount.¹
3. Casman Medium Base is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and immunological testing using pure cultures are recommended for complete identification.

Packaging

Casman Medium Base	Code No.	7123A	500 g
		7123B	2 kg
		7123C	10 kg

References

1. **Casman, E. P.** 1947. A noninfusion blood agar base for *Neisseriae*, *Pneumococci* and *Streptococci*. Am. J. Clin. Pathol. **27**:281.
2. **Casman, E. P.** 1942. J. Bacteriol. **43**:33.
3. **Casman, E. P.** 1947. J. Bacteriol. **53**:561.
4. **MacFaddin, J. D.** 1985. Media for the isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 131-143. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CDC ANAEROBE AGAR (7486)

Intended Use

CDC Anaerobe Agar is used with blood for the isolation and cultivation of fastidious, obligately anaerobic bacteria.

Product Summary and Explanation

CDC Anaerobe Agar was developed by Dowell et al. of the Center for Disease Control.^{1,2} This medium was formulated as an enriched, nonselective medium for isolation and cultivation of a wide variety of obligately anaerobic microorganisms, particularly those found in clinical materials.¹⁻³ CDC Anaerobe Agar is a Tryptic Soy Agar formulation with addition of vitamin K, hemin, cystine, agar and 5% sheep blood. Improved growth of *Bacteroides melaninogenicus*, *Fusobacterium necrophorum*, *Clostridium haemolyticum*, *Actinomyces israelii*, and *Bacteroides thetaiotaomicron* were demonstrated on this medium.^{2,3}

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal. Sodium Chloride maintains the osmotic environment. Yeast Extract is the vitamin source. L-Cystine and sheep blood constituents, Hemin and Vitamin K, provide growth factors required by certain obligate anaerobes.²⁻⁴ Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Yeast Extract.....	5 g
L-Cystine	0.4 g
Agar	20 g

Final pH: 7.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Hemin (0.005 g), sterile
Vitamin K (0.01 g), sterile

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 50 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and aseptically add 5% sterile blood and a sterile solution containing hemin (0.005 g) and vitamin K (0.01 g).

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and light beige. Prepared medium with 5% sheep blood is red and opaque.

Expected Cultural Response: Cultural response on CDC Anaerobe Agar supplemented with blood at 35°C after 48 - 72 hours incubation under anaerobic conditions.

Microorganism	Response	Reactions w Added Blood
<i>Bacteroides fragilis</i> ATCC® 25285	growth	---
<i>Clostridium perfringens</i> ATCC® 13124	growth	beta hemolysis
<i>Fusobacterium nucleatum</i> ATCC® 25586	growth	---
<i>Peptostreptococcus anaerobius</i> ATCC® 27337	growth	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion of aerobic and anaerobic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.^{5,6} For the examination of bacteria in food, refer to standard methods.

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

CDC Anaerobe Agar	Code No.	7486A	500 g
		7486B	2 kg
		7486C	10 kg

References

1. **Dowell, Jr., V. R., G. L. Lombard, F. S. Thompson, and A. Y. Armfield.** 1977. Media for isolation, characterization, and identification of obligately anaerobic bacteria. *In* CDC laboratory manual. Center for Disease Control, Atlanta, GA.
2. **Allen, S., J. A. Siders, and Marler.** 1985. *In* Lennette, Balows, Hausler, and Shadomy (eds.). Manual of clinical microbiology, 4th ed. American Society for Microbiology. Washington, D.C.
3. **Dowell, Jr., V. R., and T. M. Hawkins.** 1974. Laboratory methods in anaerobic bacteriology, CDC laboratory manual. HEW Publication No. (CDC) 79-8272. Center for Disease Control, Atlanta, GA.
4. **Starr, Killgore, and V. R. Dowell, Jr.** 1971. Appl. Microbiol. **22**:655.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CETRIMIDE AGAR (7222)

Intended Use

Cetrimide Agar is used in the isolation and identification of *Pseudomonas aeruginosa*.

Product Summary and Explanation

Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is the most frequently isolated nonfermentative bacillus in clinical specimens.¹ This organism is a significant cause of burn and nosocomial infections.² The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.¹

Pseudomonas aeruginosa produces a number of water-soluble pigments, including the yellow-green or yellow-brown fluorescent pigment pyoverdinin (fluorescein).² When pyoverdinin combines with the blue water-soluble pigment pyocyanin, the bright green color characteristic of *Pseudomonas aeruginosa* is created.² Agar containing Cetrimide has been used successfully to isolate *Pseudomonas aeruginosa* from contaminated specimens.³

King, Ward, and Raney developed Medium A (Tech Agar) to enhance the production of pyocyanin in *Pseudomonas* spp.⁴ Cetrimide Agar is prepared according to this formula with the addition of Cetrimide.⁴ Cetrimide Agar is recommended in the examination of food and in United States Pharmacopeia (USP XXIII) for use in Microbial Limit Test.^{5,6}

Principles of the Procedure

Enzymatic Digest of Gelatin provides the nitrogen, vitamins, and carbon in Cetrimide Agar. Magnesium Chloride and Potassium Chloride enhance the production of pyocyanin and fluorescein.⁷ Cetrimide (cetyltrimethylammonium bromide) is the selective agent. Cetrimide acts as a quaternary ammonium cationic detergent causing nitrogen and phosphorous to be released from bacterial cells other than *Pseudomonas aeruginosa*. Agar is the solidifying agent. Glycerol is supplemented as a source of carbon.

Formula / Liter

Enzymatic Digest of Gelatin	20 g
Magnesium Chloride	1.4 g
Potassium Chloride	10 g
Cetrimide (Cetyltrimethylammonium Bromide)	0.3 g
Agar	13.6 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement /Liter

Glycerol.....	10 g

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 45.3 g of the medium and 10 g of glycerol in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is light to moderately hazy and grey-white w/precipitate.

Expected Cultural Response: Cultural response on Cetrimide Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	inhibited	--
<i>Pseudomonas aeruginosa</i> ATCC® 10145	growth	green-yellow to blue-green colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	green-yellow to green colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate *Pseudomonas aeruginosa* colonies directly on Cetrimide Agar by the streak method from nonselective medium or the clinical specimen. When plating directly from the specimen, the inoculum level will vary.

Results

Examine plates or tubes for the presence of characteristic blue, blue-green, or yellow-green pigment. *Pseudomonas aeruginosa* typically produces both pyocyanin and fluorescein.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescein production because this yellowing does not fluoresce.⁴
3. Some nonfermenters and some aerobic spores formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.⁴
4. Studies of Lowbury and Collins⁷ showed *Ps. aeruginosa* can lose its fluorescence under UV if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.
5. Further tests are necessary for confirmation of *Ps. aeruginosa*.

Packaging

Cetrimide Agar	Code No.	7222A	500 g
		7222B	2 kg
		7222C	10 kg

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
2. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
3. **Robin, T., and J. M. Janda.** 1984. Enhanced recovery of *Pseudomonas aeruginosa* from diverse clinical specimens on a new selective agar. Diag. Microbiol. Infect. Dis. 2:207.
4. **King, E. O., M. K. Ward, and E. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
7. **Lowbury, E. J. L., and A. G. Collins.** 1955. The use of a new cetrimide product in a selective medium for *Pseudomonas aeruginosa*. J. Clin. Pathol. 8:47.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CHARCOAL AGAR (7586)

Intended Use

Charcoal Agar is used for the cultivation of fastidious microorganisms, particularly *Bordetella pertussis*.

Product Summary and Explanation

Charcoal Agar is prepared according to the method of Mishulow, Sharpe, and Cohen.¹ Charcoal Agar became an efficient substitute for Bordet-Gengou Agar in the production of *Bordetella pertussis* vaccines. This medium is used as a maintenance medium for stock cultures of *Bordetella* spp., and when supplemented with horse blood, for cultivation and isolation of *Haemophilus influenzae*.²

Bordetella consists of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. avium*.³ All *Bordetella* spp. are respiratory pathogens, residing on mucous membranes of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis.

Principles of the Procedure

The nitrogen and carbon sources are provided by Beef Heart Infusion Solids and Enzymatic Digest of Gelatin. Yeast Extract is the vitamin source in this medium. Sodium Chloride maintains the osmotic environment. Soluble Starch and charcoal absorb toxic metabolites.

Formula / Liter

Beef Heart Infusion Solids	12 g
Enzymatic Digest of Gelatin	10 g
Sodium Chloride	5 g
Soluble Starch.....	10 g
Yeast Extract.....	3.5 g
Charcoal.....	4 g
Agar	18 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 62.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and gently remix medium prior to dispensing.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and charcoal grey.

Prepared Appearance: Prepared medium is black and opaque.

Expected Cultural Response: Cultural response on Charcoal Agar at 35°C after 1 - 4 days incubation.

Microorganism	Response
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus pneumoniae</i> ATCC® 6303	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on isolation, identification, and maintenance of *Bordetella* spp. and other fastidious microorganisms.^{3,4}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.²

Packaging

Charcoal Agar	Code No.	7586A	500 g
		7586B	2 kg
		7586C	10 kg

References

1. **Mishulow, L., L. S. Sharpe, and L. L. Cohen.** 1953. Beef-heart charcoal agar for the preparation of pertussis vaccines. *Am. J. Public Health*, **43**:1466.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 110-114. Williams & Wilkins, Baltimore, MD.
3. **Marcon, M. J.** 1995. *Bordetella*, p. 566-573. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. *Clinical microbiology procedures handbook*. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CHO MEDIUM BASE (7563)

Intended Use

CHO Medium Base is used with carbohydrates for the differentiation of anaerobic bacteria on the basis of carbohydrate fermentation reactions.

Product Summary and Explanation

CHO Medium Base was developed by Dowell and Hawkins of the Center for Disease Control.¹ This medium was formulated to provide an excellent system for characterizing clostridia and other anaerobic bacteria on the basis of fermentation reactions.² CHO Medium Base is intended to be supplemented with carbohydrates.

Carbohydrate fermentation is detected by a visible color change of the medium. When a carbohydrate is metabolized by the organism, organic acids are produced and the medium becomes acidified.

Principles of the Procedure

The nitrogen, amino acids, and carbon sources are provided by Enzymatic Digest of Casein in CHO Medium Base. Yeast Extract supplies essential vitamins. Ascorbic acid, L-Cystine and Sodium Thioglycollate are added as reducing agents to create and maintain an anaerobic environment. Sodium Chloride maintains the osmotic balance of the medium. Ascorbic Acid is added to create a proper environment for organism growth. Bromthymol Blue is an indicator of pH changes in the medium. Bromthymol Blue changes the medium from grey-green to yellow when the amount of acid produced by carbohydrate fermentation is greater than alkaline end products of nitrogen degradation.³ The small amount of Agar provides a semi-solid environment and minimizes oxygen diffusion.

Formula / Liter

Enzymatic Digest of Casein	15 g
Yeast Extract.....	7 g
L-Cystine	0.25 g
Ascorbic Acid	0.1 g
Sodium Chloride	2.5 g
Sodium Thioglycollate.....	0.5 g
Bromthymol Blue.....	0.01 g
Agar	0.75 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Desired Carbohydrate, 62.5 mL
of a 10% sterile solution

Precaution

1. For Laboratory Use.

Directions

1. Suspend 26 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and aseptically add 62.5 mL of a 10% sterile solution of desired carbohydrate.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without carbohydrates is trace to slightly hazy and light grey-green.

Expected Cultural Response: Cultural response in CHO Medium Base supplemented with dextrose or lactose at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions	
		Dextrose	Lactose
<i>Bacteroides fragilis</i> ATCC® 25285	growth	positive	positive
<i>Clostridium perfringens</i> ATCC® 13124	growth	positive	positive
<i>Clostridium sporogenes</i> ATCC® 11437	growth	positive	negative
<i>Escherichia coli</i> ATCC® 25922	growth	positive	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion of aerobic and anaerobic bacteria from clinical specimens, refer to appropriate procedures outlined in the references.^{2,4} For the examination of bacteria in food, refer to standard methods.

Results

A yellow color in upper one-third or throughout the medium indicates acid production, i.e., fermentation of the carbohydrate. A grey-green color indicates that the carbohydrate has not been degraded and only the nitrogen source was utilized.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

CHO Medium Base	Code No.	7563A	500 g
		7563B	2 kg
		7563C	10 kg

References

1. **Dowell, Jr., V. R., and T. M. Hawkins.** 1977. Laboratory methods in anaerobic bacteriology, CDC laboratory manual. HEW Publication No. (CDC), 78-8272. Center for Disease Control, Atlanta, GA.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **MacFaddin, J.** 1980. Biochemical tests for identification of medical bacteria, 2nd ed. Williams & Wilkins, Baltimore, MD.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CLED AGAR (7122)

Intended Use

CLED Agar is used for the differentiation and enumeration of microorganisms in urine.

Product Summary and Explanation

CLED Agar is an abbreviation for Cystine Lactose-Electrolyte-Deficient Agar. Sandys developed an electrolyte-deficient medium that prevented *Proteus* spp. from swarming.¹ Mackey and Sandys modified the formula by substituting lactose and sucrose for mannitol, and increasing the amount of indicator and agar.² While investigating this medium for a dip slide technique, the formula was again modified omitting sucrose and adding cystine.³

CLED Agar is recommended in the spread plate technique or a dip slide for detection of bacteria in urine. This medium supports the growth of urinary pathogens and provides distinct colony morphology. CLED Agar lacks an electrolyte (salt), necessary for growth and other characteristics of certain bacteria.⁴ CLED Agar is popular in European laboratories.⁵

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Gelatin, and Beef Extract provide the nitrogen, vitamins, and carbon in CLED Agar. Lactose is the carbohydrate. L-Cystine is added as a growth supplement for cystine-dependent coliforms. Organisms capable of fermenting lactose will lower the pH and change color of the medium from green to yellow. Bromthymol Blue is the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	4 g
Enzymatic Digest of Casein	4 g
Beef Extract	3 g
Lactose	10 g
L-Cystine	0.128 g
Bromthymol Blue.....	0.02 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 36 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and light grey-green.

Expected Cultural Response: Cultural response on CLED Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	yellow colonies
<i>Proteus mirabilis</i> ATCC® 12453	growth	blue to blue- green colonies
<i>Staphylococcus aureus</i> ATCC® 25923	growth	yellow colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on collection and processing of urine cultures refer to appropriate references.⁵⁻⁷

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

CLED Agar	Code No.	7122A	500 g
		7122B	2 kg
		7122C	10 kg

References

1. **Sandys, G. H.** 1960. A new method of preventing swarming of *Proteus* spp. with a description of a new medium suitable for use in routine laboratory practice. J. Med. Lab. Technol. **17**:224.
2. **Mackey, J. P., and G. H. Sandys.** 1965. Laboratory diagnosis of infections of the urinary tract in general practice by means of a dip-inoculum transport medium. Br. Med. J. **2**:1286.
3. **Mackey, J. P., and G. H. Sandys.** 1966. Diagnosis of urinary tract infections. Br. Med. J. **1**:1173.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, MD.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

CLOSTRIDIUM DIFFICILE AGAR (7385)

Intended Use

Clostridium Difficile Agar is used with blood, cycloserine, and cefoxitin for the isolation of *Clostridium difficile*.

Product Summary and Explanation

Clostridium difficile is a major cause of antibiotic-associated diarrhea and pseudomembranous colitis.¹ It is now one of the most commonly detected enteric pathogens, and an important cause of nosocomial infections in hospitals and nursing homes.¹ The organism has been isolated from diverse natural habitats, including soil, hay, sand, dung from various large mammals, and from dog, cat, rodent and human feces.¹

In 1979, George et al. developed a medium called CCFA (cycloserine-cefoxitin-fructose agar), based on the Egg Yolk Agar formula of McClung and Toabe with fructose replacing glucose.² Clostridium Difficile Agar is a modification of the original CCFA formulation.

Principles of the Procedure

Proteose Peptone provides nitrogen, vitamins, and amino acids in Clostridium Difficile Agar. Fructose is the fermentable carbohydrate used to enhance recovery and growth of *C. difficile*. The Phosphates are buffering agents in this medium. Magnesium Sulfate is a source of inorganic ions to stimulate growth. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Horse blood provides essential growth factors in Clostridium Difficile Agar. Cycloserine and Cefoxitin are selective agents against aerobic, anaerobic, and facultatively anaerobic gram-positive and gram-negative bacteria. At the concentration of antibiotics used *C. difficile* is not inhibited significantly, while other anaerobes, including most clostridia, are inhibited.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Animal Tissue.....	15 g
Pork Brain Heart Infusion Solids.....	10 g
Fructose	6 g
Disodium Phosphate.....	5 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.1 g
Sodium Chloride	2 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Horse Blood, 7%
Cycloserine, 0.5 g/L
Cefoxitin, 0.016 g/L

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 69 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and aseptically add 7% horse blood, cycloserine (0.5 g/L) and cefoxitin (0.016 g/L).

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is light amber and trace to slightly hazy. With Supplements, the prepared agar is opaque and red.

Expected Cultural Response: Cultural response on Clostridium Difficile Agar supplemented with 7% horse blood, cycloserine, and cefoxitin at 35°C after 24-72 hours incubation.

Microorganism	Response
<i>Bacteroides fragilis</i> ATCC® 25285	inhibited
<i>Clostridium difficile</i> ATCC® 9689	growth
<i>Clostridium perfringens</i> ATCC® 13124	inhibited
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of *C. difficile* and other anaerobic bacteria refer to specific procedures in appropriate references.^{1,3}

Results

Colonies of *C. difficile* are 4 - 6 mm in diameter, irregular, raised, opaque, and grey-white after 48 hours incubation.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *C. difficile*.
2. Clostridium Difficile Agar does not contain Neutral Red indicator because it is designed for use with horse blood.²
3. Typical Gram stain morphology of *C. difficile* may not be evident in colonies picked from this medium because of antibiotics present. Subculture suspected colonies to blood agar to obtain characteristic morphology.

Packaging

Clostridium Difficile Agar	Code No.	7385A	500 g
		7385B	2 kg
		7385C	10 kg

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **George, W. L., V. L. Sutter, D. Citron, and S. M. Finegold.** 1979. Selective and differential medium for isolation of *Clostridium difficile*. J. Clin. Microbiol. 9:214.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

COLUMBIA BLOOD AGAR BASE (7125)

Intended Use

Columbia Blood Agar Base is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Columbia blood agar base media are 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, Columbia Blood Agar Base is used as a general purpose media.

Columbia Blood Agar Base was developed after the Columbia Agar formulation described by Ellner et al. from Columbia University.¹ Columbia (Blood Agar Base) BAB is specified in the Compendium of Methods for the Microbiological Examination of Foods.²

Principles of the Procedure

The nitrogen, vitamin, and carbon, sources are provided by Enzymatic Digest of Animal Tissue, Enzymatic Digest of Casein, and Yeast Enriched Peptone. Corn Starch increases growth of *Neisseria* spp., and enhances the hemolytic reactions of some streptococci. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β -hemolytic streptococci.³ Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms, and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.⁴

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	8 g
Yeast Enriched Peptone	10 g
Corn Starch.....	1 g
Sodium Chloride	5 g
Agar	14 g

Final pH: 7.3 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to skin, eyes, and mucous membranes

Directions

1. Suspend 43 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium without blood is light to medium amber, and clear to moderately hazy. With 5% sheep blood the medium is red and opaque.

Expected Cultural Response: Cultural response on Columbia Blood Agar Base at 35°C after 18 - 24 hours incubation.

Microorganism	Response (Plain and with 5% Sheep Blood)	Reactions (With 5% Sheep Blood)
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, and stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.⁴
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine the medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁵

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime-hemolysis (α') is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.⁴ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Packaging

Columbia Blood Agar Base	Code No. 7125A	500 g
	7125B	2 kg
	7125C	10 kg

References

1. **Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi.** 1966. A new culture medium for medical bacteriology. *Am. J. Clin. Pathol.* **45**:502-504.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
3. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. *Am. J. Clin. Pathol.* **17**:281-289.
4. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D. C.
5. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, *Clinical microbiology procedures handbook*, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

COLUMBIA BROTH (7127)

Intended Use

Columbia Broth is used for the cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Columbia Broth is prepared according to the formula described by Morello and Ellner.¹ During their study, Columbia Broth was developed as a medium for blood cultures, and found superior to a commonly used broth for growth of *Staphylococcus aureus*, *E. coli*, and streptococci.¹ In the presence of CO₂ and supplemented with SPS, Columbia Broth is an excellent blood culture medium and contains sufficient sulfonamide antagonists to prevent sulfonamide inhibition of growth.²

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Animal Tissue, Enzymatic Digest of Casein, Enzymatic Digest of Heart Muscle, and Yeast Enriched Peptone. Sodium Chloride maintains the osmotic balance of the medium. Dextrose is added as a carbon energy source. The medium is buffered with Tris ([hydroxymethyl] aminomethane) and Sodium Carbonate. Ferrous Sulfate and Magnesium Sulfate are added to facilitate bacterial growth. Corn Starch is omitted from Columbia Broth to reduce opalescence.¹ L-Cystine is the reducing agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Yeast Enriched Peptone	10 g
Enzymatic Digest of Heart Muscle	3 g
Sodium Chloride	5 g
Dextrose.....	2.5 g
L-Cystine	0.1 g
Magnesium Sulfate	0.1 g
Ferrous Sulfate	0.02 g
Tris (hydroxymethyl) aminomethane.....	0.83 g
Tris (hydroxymethyl) aminomethane-HCl	2.86 g
Sodium Carbonate	0.6 g
Final pH 7.3 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 35 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared broth is golden-orange to amber and clear.

Expected Cultural Response: Cultural response in Columbia Broth at 35°C after 24 - 72 hours incubation.

Microorganism	Response
<i>Bacillus fragilis</i> ATCC® 25285	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth
<i>Staphylococcus epidermidis</i> ATCC® 12228	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Process each specimen as appropriate. Refer to correct references for specific procedures.^{2,3}

Results

Examine medium for growth. Gram-negative bacilli tend to grow diffusely, Gram-positive cocci exhibit puff-ball type growth, and strict aerobes, such as pseudomonads and yeast, usually grow in a thin layer on the surface of the broth.² To ensure no growth is present, subculture inoculated and incubated Columbia Broth to appropriate non-selective media.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Opalescence in Columbia Broth cannot always be relied upon as evidence of bacterial growth.
3. It is possible for significant number of viable bacteria to be present in an inoculated and incubated blood culture bottle without the usual signs of bacterial growth.

Packaging

Columbia B	Code No	7127A	500 g
		7127B	2 kg
		7127C	10 kg

References

1. **Morello, J. A., and P. D. Ellner.** 1969. New medium for blood cultures. *Appl. Microbiol.* **17**:68-07.
2. **Isenberg, H. D. (ed.).** 1992. *Clinical microbiology procedures handbook*, vol. 1 American Society for Microbiology, Washington, D.C.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. *Manual of clinical microbiology*, 6th ed. American Society of Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

COLUMBIA CNA AGAR (7126)

Intended Use

Columbia CNA Agar is used with blood for the selective isolation of gram-positive cocci.

Product Summary and Explanation

Ellner et al. described Columbia CNA Agar as a variation of Columbia Blood Agar Base that is selective for gram-positive cocci.¹ Colistin and Nalidixic Acid are added to the formula, selecting for gram-positive organisms and fungi by suppressing gram-negative bacteria. Columbia CNA Agar is used as a primary plating medium for urine cultures.³

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Animal Tissue, Enzymatic Digest of Casein, and Yeast Enriched Peptone. Corn Starch increases growth of *Neisseria* spp., and enhances the hemolytic reactions of some streptococci. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Nalidixic Acid and Colistin are the antimicrobics suppressing the growth of *Enterobacteriaceae* and *Pseudomonas* spp., and allowing yeast, staphylococci, streptococci, and enterococci to grow.⁴ Certain gram-negative organisms, such as *Gardnerella vaginalis* and certain *Bacteriodes* spp., can grow very well on Columbia CNA Agar with blood.⁴ Colistin disrupts the cell membrane of gram-negative organisms, particularly effective against *Pseudomonas* spp.² Nalidixic Acid blocks DNA replication in susceptible bacteria and acts against many gram-negative bacteria.²

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	8 g
Yeast Enriched Peptone	10 g
Corn Starch.....	1 g
Sodium Chloride	5 g
Colistin	0.015 g
Nalidixic Acid.....	0.01 g
Agar	14 g

Final pH 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 43 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Do not overheat medium.
4. Prepare 5% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium without blood is light to moderately hazy and light to medium amber. With 5% sheep blood the medium is red and opaque.

Expected Cultural Response: Cultural response on Columbia CNA Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Pseudomonas aeruginosa</i> ATCC® 27853	inhibited	-
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate specimens directly onto surface of the medium. Streak for isolation with inoculating loop, and stab the agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.⁵
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine the medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:³

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime-hemolysis (α) is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
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.⁵
3. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.⁵ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%) in accordance with established laboratory procedures.
4. *Proteus* spp. occasionally grow on CNA Agar and may initially be confused with streptococci because of the small size of the colonies.²

Packaging

Columbia CNA Agar	Code No.	7126A	500 g
		7126B	2 kg
		7126C	10 kg

References

1. Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi. 1966. A new culture medium for medical bacteriology. *Am. J. Clin. Pathol.* **45**:502-504.
2. Estevez, E. G. 1984. Bacteriological plate media: review of mechanisms of action. *Lab. Med.* **15**:258-262.
3. Isenberg, H. D. (ed.). 1992. *Clinical microbiology procedures handbook*, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.
4. Baron, E. J., L. R. Peterson, and S. M. Finegold. 1994. *Bailey & Scott's diagnostic microbiology*, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
5. Ruoff, K. L. 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D. C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

COOKED MEAT MEDIUM (7110)

Intended Use

Cooked Meat Medium is used for the cultivation of anaerobic microorganisms.

Product Summary and Explanation

In 1890, Smith used fresh unheated animal tissue for cultivating anaerobic organisms.¹ Tarozzi confirmed Smith's findings and discovered meat broth could be heated to 104 – 105°C for 15 minutes without destroying nutrients.² A steam sterilized emulsion of brain tissue in water was employed by von Hibler.^{3,4} von Hibler found organisms in cooked brain broth were less susceptible to harmful effects of toxic metabolic products than in carbohydrate serum media.^{3,4} Robertson substituted beef heart for brain tissue and Cooked Meat Medium is prepared according to this formula.⁵

Cooked Meat Medium initiates growth from a small inoculum, important for clinical specimens. Cooked Meat Medium is recommended in standard methods for food testing.^{6,7} Cooked Meat Medium provides an effective maintenance medium. This medium can be used to differentiate saccharolytic from proteolytic *Clostridium* spp.⁸ Saccharolytic species rapidly form acid and gas without digesting meat. Proteolytic species break down meat to amino acids and form sulfur compounds (blackening and putrid smell).

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Animal Tissue and Beef Heart. The low concentration of Dextrose is sufficient as the energy source, but not high enough to accumulate toxic metabolites. Sodium Chloride maintains the osmotic balance. Solid meat particles provide favorable growth conditions for anaerobes due to reducing action of -SH (sulfhydryl) groups of muscle protein.²⁻⁴ Sulfhydryl groups are more accessible in denatured proteins, therefore use of cooked meat particles is preferred.⁸

Formula / Liter

Beef Heart..... 454 g
Enzymatic Digest of Animal Tissue..... 20 g
Dextrose..... 2 g
Sodium Chloride 5 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Place 1.25 g of meat granules into a test tube and add 10 mL of purified water.
2. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Medium is dehydrated brown pellets.

Prepared Appearance: Prepared medium is clear amber supernatant over insoluble granules.

Expected Cultural Response: Cultural response in Cooked Meat Medium at 35°C after 48 - 72 hours incubation.

Microorganism	Response
<i>Clostridium novyi</i> ATCC® 7659	growth
<i>Clostridium perfringens</i> ATCC® 13124	growth
<i>Clostridium sporogenes</i> ATCC® 11437	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate specimen deep into meat particles (bottom of the tube). Tissue specimens should be ground prior to inoculation. For a complete discussion on the isolation and identification of aerobic and anaerobic bacteria, refer to appropriate procedures.

Results

Typically growth is visually observed in media by turbidity and/or presence of gas bubbles.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if the appearance has changed from the original color or texture. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Cooked Meat Medium	Code No.	7110A	500 g
		7110B	2 kg
		7110C	10 kg

References

1. **Smith, T.** 1890. Centr. Bakteriologie. **7**:509.
2. **Tarozzi, G.** 1905. Über ein leicht in aerober Weise ausführbares Kulturmedium von einigen bis jetzt für strenge Anaerobien geeigneten Keimen. Zentralb. Bakteriologie. **38**:619.
3. **von Hübner, E.** 1899. Beiträge zur Kenntnis der durch anaerobe Spaltpilze erzeugten Infektions-Krankheiten der Tiere und des Menschen etc. Centr. Bakteriologie. **25**:513, 594, 631.
4. **von Hübner, E.** 1908. Untersuchungen über die pathogenen Anaerobier, Jena: Verlag Fischer.
5. **Robertson, M.** 1916. Notes upon certain anaerobes isolated from wounds. J. Pathol. Bacteriology. **20**:327.
6. **Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
7. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
8. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification maintenance of medical bacteria, vol. 1, p. 755-762. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

D/E NEUTRALIZING AGAR (7375)

Intended Use

D/E Neutralizing Agar is used for the isolation of microorganisms from sanitized environmental surfaces.

Product Summary and Explanation

D/E Neutralizing Agar was developed by Dey and Engley¹ to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals, including quaternary ammonium compounds, phenolics, iodine, chlorine preparations, mercurials, formaldehyde, and glutaraldehyde. D/E Neutralizing media neutralize higher concentrations of residual antimicrobials when compared with other standard neutralizing formulations, such as Lethen media, Thioglycollate media, and Neutralizing Buffer.^{2,3}

Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test. D/E Neutralizing Agar effectively neutralize the inhibitory action of disinfectant carryover,^{4,5} allowing differentiation between bacteriostasis and true bactericidal action of disinfectant chemicals. This is a critical characteristic to consider when evaluating a disinfectant. D/E Neutralizing Agar is recommended for use in disinfectant evaluations, environmental sampling (swab and contact plate methods), and testing of water-miscible cosmetics.⁶

Principles of the Procedure

Enzymatic Digest of Casein and Yeast Extract provides nitrogen, carbon, vitamins, and minerals in D/E Neutralizing Agar. Dextrose is a source of fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with lecithin, ethanol. Bromcresol Purple is used as a colorimetric indicator to demonstrate the production of acid from the fermentation of dextrose.

Formula / Liter

Enzymatic Digest of Casein	5 g
Yeast Extract.....	2.5 g
Dextrose.....	10 g
Sodium Thioglycollate	1 g
Sodium Thiosulfate	6 g
Sodium Bisulfite	2.5 g
Polysorbate 80	5 g
Lecithin (Soybean)	7 g
Bromcresol Purple	0.02 g
Agar	15 g

Final pH: 7.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. HARMFUL. May cause sensitization by inhalation. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 54 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, moist, lumpy, and blue-grey to green.

Prepared Appearance: Prepared medium is purple and opaque.

Expected Cultural Response: Cultural response in D/E Neutralizing Agar at 35°C after 40 - 48 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth
<i>Salmonella typhimurium</i> ATCC® 14082	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

D/E Neutralizing Agar is used in a variety of procedures. Consult appropriate references for complete information.⁶

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

D/E Neutralizing Agar	Code No.	7375A	500 g
		7375B	2 kg
		7375C	10 kg

References

1. **Engley, F. B., Jr. and B. P. Dey.** 1970. A universal neutralizing medium for antimicrobial chemicals. Presented at the Chemical Specialties Manufacturing Association (CSMA) Proceedings. 56th Mid-Year Meeting.
2. **Dey, B. P. and F. B. Engley, Jr.** 1983. Methodology for recovery of chemically treated *Staphylococcus aureus* with neutralizing medium. Appl. Environ. Microbiol. **45**:1533-1537.
3. **Dey, B. P., and F. B. Engley, Jr.** 1978. Environmental sampling devices for neutralization of disinfectants, presented at the 4th International Symposium on Contamination Control.
4. **Dey, B. P., and F. B. Engley, Jr.** 1994. Neutralization of antimicrobial chemicals by recovery media. J. Microbiol. Methods. **19**:51-58.
5. **Dey, B. P., and F. B. Engley, Jr.** 1995. Comparison of Dey and Engley (D/E) Neutralizing Medium to Lethen Medium and Standard Methods Medium for recovery of *Staphylococcus aureus* from sanitized surfaces. J. Ind. Microbiol. **14**:21-25.
6. **Curry, A. S., J. G. Graf, and G.N. McEwen, Jr. (eds.).** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

D/E NEUTRALIZING BROTH (7562)

Intended Use

D/E Neutralizing Broth is used for testing and neutralizing of antiseptics and disinfectants.

Product Summary and Explanation

D/E Neutralizing Broth was developed by Dey and Engley to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals,¹ including quaternary ammonium compounds, phenolics, iodine, chlorine preparations, mercurials, formaldehyde, and glutaraldehyde. D/E Neutralizing media neutralize higher concentrations of residual antimicrobials when compared with other standard neutralizing formulations, such as Lethen media, Thioglycollate media, and Neutralizing Buffer.^{2,3}

Total neutralization of disinfectants is critical. Disinfectant residues can result in a false-negative (no-growth) test. D/E Neutralizing Broth effectively neutralizes the inhibitor action of disinfectant carryover,^{4,5} allowing differentiation between bacteriostasis and the true bactericidal action of disinfectant chemicals. This is a critical characteristic to consider when evaluating a disinfectant. D/E Neutralizing Broth is recommended for use in disinfectant evaluations, environmental sampling (swab and contact plate methods), and testing of water-miscible cosmetics.⁶

Principles of the Procedure

Enzymatic Digest of Casein and Yeast Extract provide nitrogen, carbon, vitamins, and minerals in D/E Neutralizing Broth. Dextrose is a source of fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and, with Lecithin, ethanol. Bromcresol Purple is used as a colorimetric indicator to demonstrate the production of acid from the fermentation of dextrose.

Formula / Liter

Enzymatic Digest of Casein	5 g
Yeast Extract.....	2.5 g
Dextrose.....	10 g
Sodium Thioglycollate	1 g
Sodium Thiosulfate	6 g
Sodium Bisulfite	2.5 g
Lecithin.....	7 g
Bromcresol Purple	0.02 g

Final pH: 7.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / Liter

Polysorbate 80.....5 g

Precautions

1. For Laboratory Use.
2. HARMFUL. Irritating to eyes, respiratory system, and skin. May cause sensitization by inhalation.

Directions

1. Dissolve 34 g of the medium and 5 g of Polysorbate 80 in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is purple, opaque.

Expected Cultural Response: Cultural response in D/E Neutralizing Broth at 35°C after 40 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 11775	growth
<i>Salmonella typhi</i> ATCC® 19430	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Shigella flexneri</i> ATCC® 12022	growth
<i>Shigella sonnei</i> ATCC® 25931	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

D/E Neutralizing Broth is used in a variety of procedures. Consult appropriate references for complete information.⁶

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

D/E Neutralizing Broth	Code No.	7562A	500 g
		7562B	2 kg
		7562C	10 kg

References

1. Engley, F. B., Jr. and B. P. Dey. 1970. A universal neutralizing medium for antimicrobial chemicals. Presented at the Chemical Specialties Manufacturing Association (CSMA) Proceedings. 56th Mid-Year Meeting.
2. Dey, B. P. and F. B. Engley, Jr. 1983. Methodology for recovery of chemically treated *Staphylococcus aureus* with neutralizing medium. Appl. Environ. Microbiol. **45**:1533-1537.
3. Dey, B. P., and F. B. Engley, Jr. 1978. Environmental sampling devices for neutralization of disinfectants. Presented at the 4th International Symposium on Contamination Control.
4. Dey, B. P., and F. B. Engley, Jr. 1994. Neutralization of antimicrobial chemicals by recovery media. J. Microbiol. Methods. **19**:51-58.
5. Dey, B. P., and F. B. Engley, Jr. 1995. Comparison of Dey and Engley (D/E) Neutralizing Medium to Letheen Medium and Standard Methods Medium for recovery of *Staphylococcus aureus* from sanitized surfaces. J. Ind. Microbiol. **14**:21-25.
6. Curry, A. S., J. G. Graf, and G.N. McEwen, Jr. (eds.). 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DEOXYCHOLATE AGAR (7130)

Intended Use

Deoxycholate Agar is used for the isolation and differentiation of gram-negative enteric bacilli.

Product Summary and Explanation

Deoxycholate Agar was described first by Leifson for isolation of intestinal pathogens and the enumeration of intestinal pathogens in milk and water.¹ Deoxycholate Agar was an improvement over other media because chemicals, citrates and sodium deoxycholate worked well as inhibitors. This medium is used to screen *Salmonella* spp. and *Shigella* spp. from clinical specimens.²

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in Deoxycholate Agar. Differentiation of enteric bacilli is based on fermentation of Lactose. Sodium Chloride and Dipotassium Phosphate maintain the osmotic environment of the medium. Sodium Deoxycholate, Ferric Citrate, and Sodium Citrate inhibit growth of gram-positive bacteria. Neutral Red is a pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Lactose	10 g
Sodium Deoxycholate	1 g
Sodium Chloride	5 g
Dipotassium Phosphate.....	2 g
Ferric Citrate	1 g
Sodium Citrate	1 g
Neutral Red.....	0.03 g
Agar	16 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 46 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and light pink-beige.

Prepared Appearance: Prepared medium is reddish-orange and clear to trace hazy.

Expected Cultural Response: Cultural response on Deoxycholate Agar at 37°C after 40 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	colorless colonies w/ pink centers
<i>Escherichia coli</i> ATCC® 25922	growth	pink colonies / ppt
<i>Proteus mirabilis</i> ATCC® 12453	growth	pink-brown colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	colorless colonies w/ pink centers
<i>Salmonella enteritidis</i> ATCC® 13076	growth	colorless colonies
<i>Salmonella typhimurium</i> ATCC® 14082	growth	colorless colonies
<i>Shigella flexneri</i> ATCC® 12022	growth	colorless colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of enteric bacilli and Deoxycholate Agar, refer to appropriate references.

Results

Differentiation of enteric bacilli is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of Neutral Red, form pink to red colonies. Bacteria that do not ferment lactose form colorless colonies. The majority of normal intestinal bacteria ferment lactose (red colonies) while *Salmonella* spp. and *Shigella* spp. do not ferment lactose (colorless colonies).

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Deoxycholate Agar	Code No.	7130A	500 g
		7130B	2 kg
		7130C	10 kg

References

1. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. **40**:581-599.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DEOXYCHOLATE CITRATE AGAR (7186)

Intended Use

Deoxycholate Citrate Agar is used for the isolation and differentiation of gram-negative enteric bacilli.

Product Summary and Explanation

Deoxycholate Citrate Agar is a modification of Deoxycholate Agar formulated by Leifson.¹ His original formula, Deoxycholate Agar, was used for isolation of intestinal pathogens and enumeration of intestinal pathogens in milk and water.¹ Deoxycholate Agar was an improvement over other media because citrates and sodium deoxycholate worked well as inhibitors.

Leifson modified the original medium by increasing the concentration of Sodium Citrate and Sodium Deoxycholate for improved recovery of *Salmonella* spp. and *Shigella* spp. Deoxycholate Citrate Agar effectively isolates intestinal pathogens by inhibiting coliforms and many *Proteus* spp. This medium is used to screen *Salmonella* spp. and *Shigella* spp. from clinical specimens.²

Principles of the Procedure

Pork Infusion Solids and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in Deoxycholate Citrate Agar. Lactose is the fermentable carbohydrate. Sodium Deoxycholate and Sodium Citrate inhibit growth of gram-positive bacteria, coliforms and *Proteus* spp. Ferric Citrate aids in the detection of H₂S 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 bacteria produce H₂S, colonies will have black centers. The majority of normal intestinal bacteria ferment lactose and do not produce H₂S (red colonies without black centers). *Salmonella* spp. and *Shigella* spp. do not ferment lactose, but *Salmonella* may produce H₂S (colorless colonies with or without black centers). Lactose-fermenting colonies may have a zone of precipitation around them caused by the precipitation of deoxycholate in the presence of acid.

Formula / Liter

Pork Infusion Solids	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Lactose	10 g
Sodium Citrate	20 g
Ferric Citrate	1 g
Sodium Deoxycholate	5 g
Neutral Red.....	0.02 g
Agar	17 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 73 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. AVOID OVERHEATING.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pink-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, and light to medium pink- red.

Expected Cultural Response: Cultural response on Deoxycholate Citrate Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	---
<i>Escherichia coli</i> ATCC® 25922	partial inhibition	pink w/ bile precipitate
<i>Salmonella typhimurium</i> ATCC® 14082	growth	colorless colonies
<i>Shigella flexneri</i> ATCC® 12022	growth	colorless colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate specimen directly onto surface of medium. Incubate plates at 35 ± 2°C for 18 – 24 hours. Plates can be incubated for an additional 24 hours if no lactose fermentation is observed.

Results

Non-lactose fermenters 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.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Coliforms may grow on this medium, making it difficult to detect pathogens. Heavy inocula should be distributed over the entire surface of the medium to prevent complete masking of pathogens by coliforms.

Packaging

Deoxycholate Citrate Agar	Code No.	7186A	500 g
		7186B	2 kg
		7186C	10 kg

References

1. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. **40**:581-599.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DERMATOPHYTE TEST MEDIUM (7265)

Intended Use

Dermatophyte Test Medium is used for the selective isolation of dermatophytic fungi.

Product Summary and Explanation

In 1969, Taplin et al. developed this medium for the isolation and recognition of dermatophytic fungi, the causative agent of ringworm from hair, nails, and skin.^{1,2} Dermatophyte Test Medium is preferred for isolation and early recognition of *Microsporum*, *Trichophyton*, and *Epidermophyton* genera because of a distinct color change in the medium. Rapidly-growing species may produce a complete color change in the medium in 3 days. The slower-growing species will change the indicator in longer time periods. Other organisms may grow, but can be recognized as nondermatophytes by lack of a color change. A few organisms, including saprophytes, yeasts, and bacteria are capable of changing the medium from red to yellow, but are easily recognized by their distinctive colonial morphology.

Principles of the Procedure

Enzymatic Digest of Soybean Meal provides nitrogen and vitamins required for organism growth. Dextrose is included as an energy source. Phenol Red is the pH indicator used to detect acid production. Cycloheximide inhibits most saprophytic molds. The supplements, Gentamicin and Chlortetracycline, aid in selectivity of Dermatophyte Test Medium. Gentamicin inhibits Gram-negative bacteria including *Pseudomonas* spp. Chlortetracycline is a broad-spectrum antibiotic, inhibiting a wide range of Gram-positive and Gram-negative bacteria. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Soybean Meal	10 g
Dextrose.....	10 g
Phenol Red	0.2 g
Cycloheximide.....	0.5 g
Agar	20 g

Final pH: 5.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Gentamicin, 0.1 g/L
Chlortetracycline, 0.1 g/L

Precautions

1. For In Vitro Diagnostic Use.
2. VERY TOXIC. Toxic by inhalation and contact with skin. May cause harm to unborn child.

Directions

1. Suspend 40.7 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and aseptically add Gentamicin (0.1 g/L) and Chlortetracycline (0.1 g/L).

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and yellow-orange.

Expected Cultural Response: Cultural response on Dermatophyte Test Medium at 30°C after 2 - 7 days incubation.

Microorganism	Response	Reactions
<i>Aspergillus niger</i> ATCC® 16404	inhibited	---
<i>Candida albicans</i> ATCC® 10231	growth	off-white to yellow and pasty colonies
<i>Microsporum canis</i> ATCC® 36299	growth	colony exhibits pink to red reverse
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth	colony exhibits pink to red reverse
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate specimen as soon as possible after received in the laboratory. Implant cutaneous specimens by gently pressing the samples into agar surface. 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.

Results

Examine medium at 24 hours for pH indicator change in medium from yellow to red. Most pathogenic dermatophytes will produce full color change within 3 – 6 days. Certain strains of *Candida albicans* are capable of converting indicator to red, but yeasts can be recognized by their white bacteria-like appearance. Certain nondermatohyte fungi rarely can produce alkaline products (false-positive results). For definitive identification of isolates, inoculate onto conventional media.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Complete classification of dermatophytes is dependent upon microscopic observations of direct and slide culture preparations, along with biochemical and serological tests.
2. Saprophytes may redden medium if specimen material is heavily contaminated.²

Packaging

Dermatophyte Test Medium	Code No.	7265A	500 g
		7265B	2 kg
		7265C	10 kg

References

1. **Taplin D., N. Zaias, N. Rebell, and H. Blank.** 1969. Isolation and recognition of dermatophytes on a new medium (DTM). Arch. Dermatol. **99**:203.
2. **MacFaddin, J.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DEXTROSE TRYPTONE AGAR (7340)

Intended Use

Dextrose Tryptone Agar is used for isolation of mesophilic or thermophilic spoilage microorganisms from food.

Product Summary and Explanation

Dextrose Tryptone Agar evolved from research by Williams, while studying the cultivation and enumeration of thermophilic bacteria caused by “flat-sour” spoilage of canned foods.¹ In the 1930’s, the National Canners Association specified the use of Dextrose Tryptone Agar for isolating “flat sour” organisms from food products.² “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 ends of the can remain flat. *B. coagulans* is a soil microorganism, found in canned tomato products and dairy products. Conditions favorable for organism growth can result in spoilage of food products.³

Dextrose Tryptone Agar can be used to isolate other food spoilage bacteria including mesophilic, aerobic spore-formers and thermophilic “flat sour” spore-formers such as *B. stearothermophilus*.

Principles of the Procedure

Enzymatic Digest of Casein is the carbon, nitrogen, and vitamin sources used for general growth requirements in Dextrose Tryptone Agar. Dextrose is the carbohydrate source. Bromcresol Purple is the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein 10 g
Dextrose..... 5 g
Bromcresol Purple 0.04 g
Agar 15 g

Final pH: 6.7 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 30 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green-beige.

Prepared Appearance: Prepared medium is dark burgundy and clear.

Expected Cultural Response: Cultural response on Dextrose Tryptone Agar at 55°C after 24-48 hours incubation.

Microorganism	Response	Reaction
<i>Bacillus coagulans</i> ATCC® 7050	growth	yellow colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

Acid-producing organisms, such as “flat-sour” thermophiles, form yellow colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Dextrose Tryptone Agar	Code No.	7340A	500 g
		7340B	2 kg
		7340C	10 kg

References

1. **Williams, O. B.** 1936. Food Res. 1:217-221.
2. **National Canners Association.** 1933. Bacterial standards for sugar.
3. **Vanderzant, C. and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DEXTROSE TRYPTONE BROTH (7338)

Intended Use

Dextrose Tryptone Broth is used for cultivation of mesophilic or thermophilic spoilage microorganisms from food.

Product Summary and Explanation

In the 1930's, the National Canners Association specified 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 ends of the can remain flat. *B. coagulans* is a soil microorganism, and 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 Broth is similar to Dextrose Tryptone Agar, except the concentration of Tryptone and Dextrose has been doubled and Agar is removed. The American Public Health Association² and Baumgartner and Herson³ recommended this formulation for the bacteriological examination of low and medium-acid canned foods (pH 4.5 and above).

Principles of the Procedure

Enzymatic Digest of Casein is the carbon, nitrogen, and vitamin sources used for general growth requirements in Dextrose Tryptone Broth. Dextrose is the carbohydrate source. Bromcresol Purple is the pH indicator.

Formula / Liter

Enzymatic Digest of Casein 20 g
Dextrose..... 10 g
Bromcresol Purple 0.04 g
Final pH: 6.7 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 30 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green-beige.

Prepared Appearance: Prepared medium is dark burgundy and clear.

Expected Cultural Response: Cultural response in Dextrose Tryptone Broth at 55°C after 24 - 48 hours incubation.

Microorganism	Response	Reaction
<i>Bacillus stearothermophilus</i> ATCC® 12980	growth	yellow

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

Organisms that produce acid from dextrose, such as *Bacillus stearothermophilus* and other “flat-sour” organisms, are detected by the color change of the medium from dark burgundy to yellow.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Dextrose Tryptone Broth	Code No.	7338A	500 g
		7338B	2 kg
		7338C	10 kg

References

1. **National Canners Association.** 1933. Bacterial standards for sugar.
2. **Vanderzant, C. and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Baumgartner, J. G. and A. C. Herson.** 1956. Canned foods. 4th ed. Churchill Ltd. London, England.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DICHLORAN GLYCEROL (DG-18) AGAR BASE (7592)

Intended Use

Dichloran Glycerol (DG-18) Agar Base is used for the selective isolation and enumeration of yeasts and molds from foods.

Product Summary and Explanation

Dichloran Glycerol Agar Base is based on the formulation by Hocking and Pitt.¹ This medium is recommended for enumeration and isolation of yeasts and molds from dried and semi-dried foods, including fruits, spices, cereals, nuts, meat, and fish products. The highly selective composition of this medium allows for enumeration of fungal growth. In a comparative study between DG-18 Agar Base and DRBC Agar, greater recovery of two molds commonly isolated in high numbers from dried foods grew poorly on DRBC Agar.¹

A modification of DG-18 Agar Base, enhanced with Triton-X, is described as increasing inhibition of vigorously-spreading fungi.²

Principles of the Procedure

Peptone provides nitrogen and vitamins required for organism growth. Glucose is included as an energy source. Monopotassium Phosphate is a buffering agent. Magnesium Sulfate, Zinc Sulfate, and Copper Sulfate are inorganic salts used to stimulate fungal growth and sporulation. The antifungal agent, Dichloran, inhibits fungi from spreading and restricts colony size. Chloramphenicol inhibits growth of bacteria present in environmental and food samples. Chlortetracycline is a broad-spectrum antibiotic, inhibiting a wide range of gram-positive and gram-negative bacteria. Agar is the solidifying agent. Glycerol is added as a carbon source.

Formula / Liter

Peptone.....	5 g
Glucose.....	10 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Zinc Sulfate	0.01 g
Copper Sulfate	0.005 g
Dichloran.....	0.002 g
Chloramphenicol.....	0.05 g
Chlortetracycline	0.05 g
Agar	15 g

Final pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Glycerol, 220 mL

Precautions

1. For Laboratory Use.
2. VERY TOXIC. Toxic by inhalation and contact with skin, respiratory system, and digestive tract.

Directions

1. Suspend 31.6 g of the medium and 220 mL of glycerol in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and beige.

Expected Cultural Response: Cultural response on DG-18 Agar Base at 25°C after up to 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Bacillus subtilis</i> ATCC® 9372	inhibited
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references in standard methods for applications using DG-18 Agar Base for yeast and mold testing.

Results

Observe and record number of yeasts and/or molds present. Report as appropriate per/sample being tested.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Complete classification of yeast and molds is dependent upon microscopic observations of direct and/or slide culture preparations, along with biochemical and serological tests.
2. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Dichloran Glycerol (DG-18) Agar Base	Code No.	7592A	500 g
		7592B	2 kg
		7592C	10 kg

References

1. Hocking, A. D., and J. I. Pitt. 1980. J. Appl. & Env. Microbiol. **39**:488-492.
2. Beuchat, L. R., and C. A. Hwang. 1996. Int. J. Food Microbiol. **29**:161-166.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DIPEPTONE (7183)

Intended Use

Dipeptone is a blend of an enzymatic digest of animal tissue and an enzymatic digest of casein for use in preparing microbiological culture media.

Product Summary and Explanation

Dipeptone contains many peptide sizes in combination with vitamins, nucleotides, minerals, and other carbon sources. Dipeptone is particularly well suited in supplying growth requirements of fastidious bacteria. This peptone is extremely valuable in media for cultivation of pathogenic fungi. Growth of these microorganisms is rapid and colony formation is uniform and typical.

Principles of the Procedure

Dipeptone provides nitrogen, vitamins, carbon, and amino acids in microbiological culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and light beige to tan.

Prepared Appearance (2% wt/vol): Prepared medium is clear, gold with no or a light precipitate.

pH (2% Solution at 25°C): 6.8 - 7.2

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to good growth

Test Procedure

Refer to appropriate references for specific procedures using Dipeptone. For a complete discussion on fastidious organisms, refer to procedures outlined in the references.^{1,2}

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Dipeptone at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dipeptone should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to Dipeptone in its intact container when stored as directed.

Packaging

Dipeptone	Code No.	7183A	500 g
		7183B	2 kg
		7183C	10 kg

References

1. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. Vol. 1. American Society for Microbiology, Washington, D.C.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DNase TEST AGAR (7129)

Intended Use

DNase Test Agar is used for the differentiation of microorganisms on the basis of deoxyribonuclease activity.

Product Summary and Explanation

In 1956, Weckman and Catlin showed a correlation between increased DNase activity of *Staphylococcus aureus* and positive coagulase activity.¹ Their research suggested DNase activity could be used to identify potentially pathogenic staphylococci. DiSalvo confirmed their results by obtaining excellent correlation between coagulase and DNase activity of staphylococci isolated from clinical specimens.² Jeffries, Holtman, and Guse incorporated DNA in agar to study DNase production by bacteria and fungi.³ Polymerized DNA precipitates in the presence of 1N HCl, creating an opaque medium. Organisms that degrade DNA produce a clear zone around an inoculum streak. Fusillo and Weiss studied calcium requirements of staphylococci for DNase production, and concluded additional calcium was unnecessary when a complete nutritive medium was used.⁴

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue. Sodium Chloride provides essential ions while maintaining osmotic balance. Deoxyribonucleic Acid enables the detection of DNase that depolymerize DNA. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Deoxyribonucleic Acid.....	2 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light amber, clear to slightly hazy.

Expected Cultural Response: Cultural response on DNase Test Agar at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions (DNase)
<i>Serratia marcescens</i> ATCC® 8100	---	positive
<i>Staphylococcus aureus</i> ATCC® 25923	---	positive
<i>Staphylococcus epidermidis</i> ATCC® 12228	---	negative
<i>Streptococcus pyogenes</i> ATCC® 19615	---	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate plates by spotting or streaking a heavy inoculum of test organism. Use a spot approximately 5 mm in diameter or a 1 – 2 cm streak approximately 5 mm wide.
2. Incubate plates at 35 ± 2°C for 18 – 24 hours and up to 48 hours.
3. Flood plates with 1 N HCl.
4. Observe for clearing around the spot or streak. Record results.

Results

A zone of clearing around the spot or streak indicates DNase activity.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Composition of the medium, degree of aeration, pH temperature, and incubation period are important factors influencing DNase activity in culturing and testing staphylococci.⁵

Packaging

DNase Test Agar	Code No.	7129A	500 g
		7129B	2 kg
		7129C	10 kg

References

1. **Weckman, B. G., and B. W. Catlin.** 1957. Deoxyribonuclease activity of micrococci from clinical sources. J. Bacteriol. **73**:747-753.
2. **DiSalvo, J. W.** 1958. Deoxyribonuclease and coagulase activity of micrococci. Med. Tech. Bull. U. S. Armed Forces Med. J. **9**:191.3.
3. **Jeffries, C. D., D. F. Holtman, and D. G. Guse.** 1957. Rapid method of determining the activity of microorganisms on nucleic acid. J. Bacteriol. **73**:590-591.
4. **Fusillo, M. H., and D. L. Weiss.** 1959. Qualitative estimation of staphylococcal deoxyribonuclease. J. Bacteriol. **78**:520.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 275-284. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DNase TEST AGAR w/ METHYL GREEN (7260)

Intended Use

DNase Test Agar w/ Methyl Green is used for the differentiation of microorganisms on the basis of deoxyribonuclease activity.

Product Summary and Explanation

In 1956, Weckman and Catlin showed a correlation between increased DNase activity of *Staphylococcus aureus* and positive coagulase activity.¹ Their research suggested DNase activity could be used to identify potentially pathogenic staphylococci. DiSalvo confirmed their results by obtaining excellent correlation between coagulase and DNase activity of staphylococci isolated from clinical specimens.² Jeffries, Holtman, and Guse incorporated DNA in agar to study DNase production by bacteria and fungi.³ Polymerized DNA precipitates in the presence of 1N HCl, creating an opaque medium. Organisms that degrade DNA produce a clear zone around an inoculum streak. Fusillo and Weiss studied calcium requirements of staphylococci for DNase production, and concluded additional calcium was unnecessary when a complete nutritive medium was used.⁴

Kurnick discovered Methyl Green combines with highly polymerized DNA at pH 7.5.⁵ When combination does not occur, color fades, creating a clear zone around the growth. Applying this principle, Smith, Hancock, and Rhoden modified DNase Test Agar, adding Methyl Green to detect staphylococci, streptococci, and *Serratia* spp.⁶

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue. Sodium Chloride provides essential ions while maintaining osmotic balance. Deoxyribonucleic Acid enables detection of DNase that depolymerize DNA. Methyl Green is a colorimetric indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Chloride	5 g
Deoxyribonucleic Acid.....	2 g
Methyl Green.....	0.05 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and light green tan.

Prepared Appearance: Prepared medium is blue-green, clear to slightly hazy.

Expected Cultural Response: Cultural response on DNase Test Agar w/ Methyl Green at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions (DNase)
<i>Serratia marcescens</i> ATCC® 8100	---	positive
<i>Staphylococcus aureus</i> ATCC® 25923	---	positive
<i>Staphylococcus epidermidis</i> ATCC® 12228	---	negative
<i>Streptococcus pyogenes</i> ATCC® 19615	---	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate plates by spotting or streaking a heavy inoculum of test organism. Use a spot approximately 5 mm in diameter or a 1 – 2 cm streak approximately 5 mm wide.
2. Incubate plates at 35 ± 2°C for 18 – 24 hours and up to 48 hours.
3. Observe for clearing around the spot or streak. Record results.

Results

A decolorized zone (halo) around the spot or streak indicates DNase activity.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Composition of the medium, degree of aeration, pH temperature, and incubation period are important factors influencing DNase activity in culturing and testing staphylococci.⁷

Packaging

DNase Test Agar w/ Methyl Green	Code No.	7260A	500 g
		7260B	2 kg
		7260C	10 kg

References

1. Weckman, B. G., and B. W. Catlin. 1957. Deoxyribonuclease activity of micrococci from clinical sources. J. Bacteriol. **73**:747-753.
2. DiSalvo, J. W. 1958. Deoxyribonuclease and coagulase activity of micrococci. Med. Tech. Bull. U. S. Armed Forces Med. J. **9**:191.
3. Jeffries, C. D., D. F. Holtman, and D. G. Guse. 1957. Rapid method of determining the activity of microorganisms on nucleic acid. J. Bacteriol. **73**:590-591.
4. Fusillo, M. H., and D. L. Weiss. 1959. Qualitative estimation of staphylococcal deoxyribonuclease. J. Bacteriol. **78**:520.
5. Kurnick, N. B. 1950. The determination of deoxyribonuclease activity by methyl green: application to serum. Arch. Biochem. **29**:41.
6. Smith, P. B., G. A. Hancock, and D. L. Rhoden. 1969. Improved medium for detecting deoxyribonuclease-producing bacteria. Appl. Microbiol. **18**:991.
7. MacFaddin, J. D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 275-284. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

DRBC AGAR (7591)

Intended Use

DRBC Agar is used for the selective isolation and enumeration of yeasts and molds from foods.

Product Summary and Explanation

DRBC Agar is based on Dichloran Rose Bengal Chlortetracycline (DRBC) 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 proposed by King, Hocking, and Pitt.² DRBC Agar is a selective medium, supporting good growth of yeasts and molds.

Principles of the Procedure

Peptone provides nitrogen, carbon, and vitamins required for organism growth. Glucose is included as an energy source. Monopotassium Phosphate is a buffering agent. Magnesium Sulfate is a source of divalent cations and sulfate. The antifungal agent, Dichloran, is added to reduce colony diameters of spreading fungi. The pH of the medium is reduced from 7.2 to 5.6 for improved inhibition of spreading fungi.¹ Rose Bengal suppresses growth of bacteria and restricts the size and height of colonies of more rapidly growing molds. The concentration of Rose Bengal is reduced from 50 µg/mL to 25 µg/mL, found in Rose Bengal Chloramphenicol Agar, for optimal performance with Dichloran. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples. Inhibition of bacterial growth and the restricted spreading of rapidly growing molds aids in isolation of slow-growing fungi. In addition, Rose Bengal is absorbed by yeast and mold colonies, allowing 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 / Liter

Enzymatic Digest of Animal Tissue.....	5 g
Glucose.....	10 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Rose Bengal	0.025 g
Dichloran.....	0.002 g
Chloramphenicol.....	0.1 g
Agar	15 g

Final pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. VERY TOXIC. Toxic by inhalation and contact with skin.

Directions

1. Suspend 31.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pink-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and bright pink.

Expected Cultural Response: Cultural response on DRBC Agar at 25 - 30°C after 2 - 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Bacillus subtilis</i> ATCC® 9372	inhibited
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Mucor racemosus</i> ATCC® 42647	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

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 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.

Results

Colonies of mold and yeast should be apparent within 5 days incubation. Colonies of yeast appear pink because of the absorption of Rose Bengal. Report results as colony forming units per gram or milliliter of sample.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

DRBC Agar	Code No.	7591A	500 g
		7591B	2 kg
		7591C	10 kg

References

1. **King, A. D., A. D. Hocking, and J. I. Pitt.** 1979. Dichloran-rose bengal medium for the enumeration and isolation of molds from foods. *Appl. Environ. Microbiol.* **37**:959-964.
2. **Mislivec, P. B., L. R. Beuchat, and M. A. Cousin.** 1992. Yeasts and molds, p. 239-249. *In* C. Vanderzant, and D. F. Splittstoesser, (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Vanderzant, C. and D. F. Splittstoesser, (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

EC MEDIUM (7206)

Intended Use

EC Medium is used for the detection of coliform bacteria at 37°C and *Escherichia coli* at 44.5°C.

Product Summary and Explanation

EC Medium was developed by Hajna and Perry¹ in an effort to improve the methods for the detection of the coliform group and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts Mixture. Growth of spore-forming bacteria and fecal streptococci is inhibited by the bile salts, while growth of *E. coli* is enhanced by its presence. This medium can be used at 37°C for the detection of coliform organisms or at 45.5°C for the isolation of *E. coli*.

EC Medium is employed in elevated-temperature tests for distinguishing organisms of the total coliform group that also belong to the fecal coliform group.² The fecal coliform test, using EC Medium, is applicable to investigations of drinking water, stream pollution, raw water sources, wastewater treatment systems, bathing waters, seawaters, and general water-quality monitoring. Prior enrichment in presumptive media is required for optimum recovery of fecal coliforms when using EC Medium. EC Medium is used in methods for food and water testing.²⁻⁴

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, vitamins, and amino acids in EC Medium. Lactose is the carbon source. Bile Salts Mixture is the selective agent against gram-positive bacteria, particularly bacilli and fecal streptococci. Dipotassium Phosphate and Monopotassium Phosphate are the buffering agents. Sodium Chloride maintains the osmotic balance of the medium.

Formula / Liter

Enzymatic Digest of Casein 20 g
Lactose 5 g
Bile Salts Mixture 1.5 g
Dipotassium Phosphate 4 g
Monopotassium Phosphate 1.5 g
Sodium Chloride 5 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 37 g of the medium in one liter of purified water.
2. Heat to boiling to completely dissolve the medium.
3. Distribute into tubes containing inverted fermentation Durham tubes. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium gold and clear.

Expected Cultural Response: Cultural response in EC Medium at 44.5°C after 24 ± 2 hours incubation.

Microorganism	Response	Reactions (Gas)
<i>Enterobacter aerogenes</i> ATCC® 13048	poor growth	negative
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	negative
<i>Escherichia coli</i> ATCC® 25922	good growth	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using EC Medium.²⁻⁴

Results

Gas production with growth in EC Medium within 24 hours or less is considered a positive fecal coliform reaction. Failure to produce gas with little or no growth is a negative reaction.²

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

EC Medium	Code No.	7206A	500 g
		7206B	2 kg
		7206C	10 kg

References

1. **Hajna and Perry.** 1943. Am J. Public Health. **33**:550.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed., American Public Health Association, Washington, D.C.
3. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed., AOAC International, Gaithersburg, MD.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

EC MEDIUM w/ MUG (7361)

Intended Use

EC Medium w/ MUG is used for the fluorogenic detection of *Escherichia coli*.

Product Summary and Explanation

EC Medium was developed by Hajna and Perry¹ in an effort to improve the methods for the detection of the coliform group and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts Mixture. Growth of spore-forming bacteria is inhibited by the bile salts, while growth of *E. coli* is enhanced by its presence. EC Medium w/ MUG is the same formula as EC Medium, with the addition of 4-methylumbelliferyl- β -D-glucuronide. Feng and Hartman² 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 μ g/mL. Moburg³ determined the amount of MUG could be reduced to a final concentration of 50 μ g/mL without adversely affecting results.

EC Medium w/ MUG is prepared according to the formula specified by US EPA⁴ and methods for water and food testing.^{5,6}

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, vitamins, and amino acids in EC Medium w/ MUG. Lactose is the carbon source. Bile Salts Mixture is the selective agent against non-fecal gram-positive bacteria. Dipotassium Phosphate and Monopotassium Phosphate are the buffering agents. Sodium Chloride maintains the osmotic balance of the medium. Incubation at 44.5°C provides additional selectivity.

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, along with growth response and gas production, to determine the presence of *E. coli* in food and environmental samples.

Formula / Liter

Enzymatic Digest of Casein	20 g
Lactose	5 g
Bile Salts Mixture	1.5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g
4-Methylumbelliferyl- β -D-Glucuronide	0.05 g

Final pH: 6.9 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 37 g of the medium in one liter of purified water.
2. Prepare double strength broth for evaluating 10 mL samples.
3. Distribute into tubes containing inverted fermentation Durham tubes. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is light to medium gold and clear.

Expected Cultural Response: Cultural response in EC Medium w/ MUG at 44.5°C after 24 ± 2 hours incubation.

Microorganism	Response	Reactions	
		Gas	Fluorescence
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	negative	negative
<i>Escherichia coli</i> ATCC® 25922	good growth	positive	positive
<i>Klebsiella pneumoniae</i> ATCC® 33495	marked to completely inhibited	variable	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using EC Medium w/ MUG.⁴⁻⁶

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 produce gas, but will not exhibit fluorescence.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Strains of *E. coli* that fail to grow in EC Medium w/ MUG, fail to produce gas, or fail to produce glucuronidase may infrequently be encountered. Strains of *Salmonella*, *Shigella* and *Yersinia* that glucuronidase may be encountered. These strains must be distinguished from *E. coli* on the basis of other parameters, e.g., gas production, growth at 44.5°C.

Packaging

EC Medium w/ MUG	Code No.	7361A	500 g
		7361B	2 kg
		7361C	10 kg

References

1. Hajna and Perry. 1943. Am J. Public Health. **33**:550.
2. Feng, P. C. S., and P. A. Hartman. 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43**:1320-1329.
3. Moberg, L. J. 1985. Fluorogenic assay for rapid detection of *Escherichia coli* in food. Appl. Environ. Microbiol. **50**:1383-1387.
4. Federal Register. 1991. National primary drinking water regulation; analytical techniques; coliform bacteria. Fed. Regist. **56**:636-643.
5. Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 1995. Standard methods for the examination of water and wastewater, 19th ed., American Public Health Association, Washington, D.C.
6. Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

EC MEDIUM, MODIFIED (7506)

Intended Use

EC Medium, Modified is used with novobiocin for the selective pre-enrichment of *Escherichia coli* O157:H7.

Product Summary and Explanation

EC Medium was developed by Hajna and Perry¹ in an effort to improve the methods for the detection of the coliform group and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts Mixture. Growth of spore-forming bacteria and fecal streptococci were inhibited by the bile salts. EC Medium, Modified with the addition of novobiocin was first described by Okrend and Rose.² Okrend and Rose modified EC Medium by reducing the Bile Salts Mixture concentration to 1.12% and adding 20 mg/L of sodium novobiocin. Okrend and Rose et al. reported this formulation, which they called Modified EC & Novobiocin (mEC&N), was beneficial in the enrichment and detection of *E. coli* O157:H7 from meats and poultry, and is currently recommended by the U.S.D.A.³⁻⁵

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, vitamins and amino acids in EC Medium, Modified. Lactose is the carbon source. Bile Salts Mixture is a selective agent used to inhibit some gram-positive cocci and sporeformers. Novobiocin is added as a supplement to suppress the growth of nuisance organisms commonly found in food. Dipotassium Phosphate and Monopotassium Phosphate are the buffering agents. Sodium Chloride maintains the osmotic balance of the medium.

Formula / Liter

Enzymatic Digest of Casein	20 g
Lactose	5 g
Bile Salts Mixture	1.12 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g
Final pH: 6.9 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobial / 10 mL

Novobiocin, 20 mg, filter sterilized aqueous solution

Precautions

1. For Laboratory Use.
2. IRRITANT Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 36.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to room temperature and add 10 mL of a filter sterilized aqueous solution containing 20 mg of novobiocin.
5. Dispense aseptically into sterile tubes containing an inverted fermentation Durham tube.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and very light beige.

Prepared Appearance: Prepared medium is clear to trace hazy and yellow gold to amber.

Expected Cultural Response: Cultural response in EC Medium, Modified at $35 \pm 0.2^{\circ}\text{C}$ after 24 hours incubation.

Microorganism	Response	Reaction (Gas)
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	----
<i>Escherichia coli</i> ATCC® 35150	good growth	positive
<i>Escherichia coli</i> ATCC® 43894	good growth	positive
<i>Escherichia coli</i> ATCC® 43895	good growth	positive
<i>Pseudomonas aeruginosa</i> ATCC® 27853	partial – complete inhibition	----

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures on the samples being tested with EC Medium, Modified.

Results

All presumptive positive isolates should be further tested through biochemical and serologic procedures to confirm the presence of *E. coli* O157:H7.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

EC Medium, Modified	Code No.	7506A	500 g
		7506B	2 kg
		7506C	10 kg

References

1. **Hajna and Perry.** 1943. Am J. Public Health. **33**:550.
2. **Okrend, A. J. G., and B. E. Rose.** 1989. USDA Communication No. 38, rev. 4. USDA, Washington, D. C.
3. **Okrend, A. J. G., B. E. Rose, and B. Bennett.** 1990. J. Food Prot. **53**:249-252.
4. **Okrend, A. J. G., B. E. Rose, and C. P. Lattuada.** 1990. J. Food Prot. **53**:941-943.
5. **Okrend, A. J. G., B. E. Rose, and R. Matner.** 1990. J. Food Prot. **53**:936-940.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

ELLIKER BROTH (7395)

Intended Use

Elliker Broth is used for the cultivation of dairy strains of streptococci and lactobacilli.

Product Summary and Explanation

Elliker Broth is prepared according to the formulation of Elliker, Anderson, and Hannesson,¹ and modified by McLaughlin.² This slightly acidic medium contains nutrients to support the growth of streptococci and lactobacilli.

Testing for lactic acid bacteria in dairy products may be useful for various reasons.³ These include determining cause of acid defects in dairy products, evaluating lactic starter cultures, and controlling the quality of cured cheese, cultured milks, and uncultured products.³ Lactic acid bacteria found in dairy products are primarily *Streptococcus*, *Lactococcus*, *Leuconostoc*, and *Lactobacillus*.³

Principles of the Procedure

Enzymatic Digest of Casein and Gelatin are the carbon, nitrogen, and vitamin sources used for general growth requirements in Elliker Broth. Yeast Extract provides essential vitamins. Dextrose, Lactose, and Sucrose are the fermentable carbohydrates. Sodium Chloride maintains the osmotic balance of the medium. Sodium Acetate is a selective agent against gram-negative bacteria. Ascorbic Acid is added to create a reduced environment for organism growth.

Formula / Liter

Enzymatic Digest of Casein	20 g
Gelatin.....	2.5 g
Yeast Extract.....	5 g
Dextrose.....	5 g
Lactose	5 g
Sucrose	5 g
Sodium Chloride	4 g
Sodium Acetate.....	1.5 g
Ascorbic Acid	0.5 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 48.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is dark amber and clear.

Expected Cultural Response: Cultural response in Elliker Broth at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Lactobacillus casei</i> ATCC® 393	growth
<i>Lactobacillus fermentum</i> ATCC® 9338	growth
<i>Lactobacillus plantarum</i> ATCC® 8014	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on isolation and identification of streptococci and lactobacilli, refer to standard methods in food testing.³⁻⁵

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

Packaging

Elliker Broth	Code No.	7395A	500 g
		7395B	2 kg
		7395C	10 kg

References

1. **Elliker, P. R., A. W. Anderson, and G. Hannesson.** 1956. An agar culture medium for lactic acid streptococci and lactobacilli. J. Dairy Sci. **39**:1611.
2. **McLaughlin, C. B.** 1946. Readily prepared medium for cultivation of lactobacilli. J. Bacteriol. **51**:560.
3. **Frank, J. F., G. L. Christen, and L. B. Bullerman.** 1993. Test for groups of microorganisms, p. 271-286. In R. T. Marshall (ed.). Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological association of food, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

EOSIN METHYLENE BLUE AGAR (7134) (Holt, Harris & Teague)

Intended Use

Eosin Methylene Blue Agar (Holt, Harris & Teague) is used for the isolation and differentiation of Gram-negative enteric bacilli.

Product Summary and Explanation

Eosin Methylene Blue Agar (EMB) was developed by Holt-Harris and Teague.¹ This formula contains lactose and sucrose with two indicator dyes, Eosin Y and Methylene Blue. The use of Eosin Y and Methylene Blue as indicators produced sharp and distinct differentiation between colonies of lactose fermenting and nonfermenting organisms. Sucrose is included to detect coliforms that ferment sucrose more readily than lactose. EMB Agar is selective due to the presence of an inhibitor and differential based on the ability of some organisms to ferment carbohydrates with the absorption of an Eosin Y and Methylene Blue complex.

EMB Agar is recommended for use in examining clinical specimens for enteric pathogens.^{2,3}

Principles of the Procedure

Enzymatic Digest of Gelatin is the nitrogen source in EMB Agar. Lactose and Sucrose are the fermentable carbohydrates. Dipotassium Phosphate is the buffer. Eosin Y and Methylene Blue are dyes that combine to form a complex at an acid pH. At a sufficiently low pH, strong lactose fermenters such as *Escherichia coli* produce colonies with a green metallic sheen. These dyes act as both a pH indicator and an inhibitor. Gram-positive bacteria are partially inhibited on the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin.....	10 g
Lactose	5 g
Sucrose.....	5 g
Dipotassium Phosphate.....	2 g
Eosin Y.....	0.4 g
Methylene Blue	0.065 g
Agar	13.5 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Inhalation of powder may cause respiratory irritation.

Directions

1. Suspend 36 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and red-purple.

Prepared Appearance: Prepared medium is dark red to blue-purple, with or without a fine precipitate.

Expected Cultural Response: Cultural response on EMB Agar, at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	blue-black colonies with black center
<i>Enterococcus faecalis</i> ATCC® 29212	partial inhibition	colorless, pinpoint colonies
<i>Escherichia coli</i> ATCC® 25922	growth	blue-black colonies, with green metallic sheen
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	colorless, may have irregular colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	colorless

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For isolation of enteric pathogens from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of EMB Agar. Streak for isolation to permit development of discrete colonies. Incubate plates at 35°C. Examine plates at 24 and 48 hours for colonies with characteristic morphologies associated with potential pathogens.

Results

Colonies of *Salmonella* spp. and *Shigella* spp. are translucent, amber colored or colorless. Coliforms that use lactose and/or sucrose produce blue-black colonies with dark centers and a green metallic sheen. Other coliforms such as *Enterobacter* spp. form mucoid, pink colonies. Strains of *Enterococcus faecalis* are partially inhibited on this medium and are colorless pinpoint or very small colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Some strains of *Salmonella* and *Shigella* may not grow on EMB Agar.⁴
2. EMB Agar is moderately inhibitory. Some staphylococci, streptococci and yeast may grow. They will appear as small, pinpoint colonies.
3. Sterilization reduces Methylene Blue, leaving the medium orange in color. The normal blue-purple color of the medium may be restored by gently mixing.
4. Not all strains of *E. coli* produce a green metallic sheen. The presence of the green metallic sheen is not diagnostic for *E. coli*.⁴

Packaging

Eosin Methylene Blue Agar (Holt, Harris & Teague)	Code No.	7134A	500 g
		7134B	2 kg
		7134C	10 kg

References

1. **Holt-Harris, J. E., and O. Teague.** 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools. J. Infect. Dis. **18**:596.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Pezzlo, M.** 1992. Aerobic bacteriology, p. 1.0.1 – 1.20.47. In H. D. Isenberg (ed.). Clinical microbiology procedures handbook, vol. 1., American Society for Microbiology, Washington, D.C.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

EOSIN METHYLENE BLUE AGAR, LEVINE (7103)

Intended Use

Eosin Methylene Blue Agar, Levine is used for the isolation and differentiation of gram-negative enteric bacilli.

Product Summary and Explanation

Eosin Methylene Blue Agar, abbreviated EMB, was developed by Holt-Harris and Teague.¹ This formula contains lactose and sucrose with two indicator dyes, Eosin Y and Methylene Blue. Levine modified the formula by removing sucrose and doubling the concentration of lactose.^{2,3} Eosin Methylene Blue Agar, Levine is used for testing clinical materials, food, and dairy products.⁴⁻⁸ This medium is primarily used for the detection and confirmation of coliforms.

Principles of the Procedure

Enzymatic Digest of Gelatin is the nitrogen source in EMB Agar, Levin. Lactose is the carbohydrate and Dipotassium Phosphate is the buffer. Eosin Y and Methylene Blue are the indicators. Methylene Blue is also a selective agent. During strong acidic conditions, the dyes impart a metallic sheen to certain lactose fermenters, such as *E. coli*.

Formula / Liter

Enzymatic Digest of Gelatin.....	10 g
Lactose	10 g
Dipotassium Phosphate.....	2 g
Eosin Y.....	0.4 g
Methylene Blue	0.065 g
Agar	15 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 37.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light red-purple.

Prepared Appearance: Prepared medium is clear to slightly hazy and dark blue purple.

Expected Cultural Response: Cultural response on EMB Agar, Levin at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Enterococcus faecalis</i> ATCC® 29212	partial inhibition, colorless colonies
<i>Escherichia coli</i> ATCC® 25922	good growth, blue-black colonies with green metallic sheen
<i>Salmonella typhimurium</i> ATCC® 14028	good growth, colorless to amber colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Eosin Methylene Blue Agar, Levine.⁴⁻⁷

Results

Colonies of lactose fermenters are blue-black with or without a green metallic sheen. *E. coli* colonies typically are dark centered and usually have a green metallic sheen. Colonies of non-lactose fermenting bacteria are colorless and translucent. Refer to appropriate references for specific results and biochemical reactions.⁴⁻⁷

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Eosin Methylene Blue Agar, Levine	Code No.	7103A	500 g
		7103B	2 kg
		7103C	10 kg

References

1. **Holt-Harris, J. E., and O. Teague.** 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools. J. Infect. Dis. **18**:596.
2. **Levin, M.** 1918. Differentiation of *E. coli* and *A. aerogenes* on a simplified eosin-methylene blue agar. J. Infect. Dis. **23**:43-47.
3. **Levin, M.** 1921. Bacteria fermenting lactose, the significance in water analysis. Bull. 62. Iowa State College Eng. Exp. Sta., Ames, Iowa.
4. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
5. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.
7. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

EUGONIC AGAR (7135)

Intended Use

Eugonic Agar is used for the cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Eugonic Agar is prepared according to the formula described by Vera.¹ Eugonic Agar was developed to obtain eugonic (luxuriant) growth of fastidious microorganisms, and can be used with or without enrichment.² Eugonic Agar supports the growth of pathogenic fungi, including *Nocardia*, *Histoplasma*, and *Blastomyces* when enriched with blood. Niven reported the use of Eugonic Agar for the detection of lactic acid in cured meats.³ Harrison and Hansen⁴ employed the medium for plate counts, and Frank⁵ researched Eugonic Agar for germinating anaerobic spores pasteurized at 104°C.

Eugonic Agar is specified in standard methods for food testing.⁶

Principles of the Procedure

The nitrogen, amino acids, and vitamins are provided by Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal in Eugonic Agar. Sodium Chloride maintains the osmotic balance of the medium. The high concentration of Dextrose is the energy source for rapid growth of bacteria. Sodium Sulfite and L-Cystine are added to stimulate growth. The high carbohydrate content along with high sulfur (cystine) content improves growth with chromogenicity.² Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5.5 g
Sodium Chloride	4 g
Dextrose.....	5 g
Sodium Sulfite.....	0.2 g
L-Cystine	0.3 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 45 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. If desired, prepare 5 to 10% blood agar by adding appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and light amber.

Expected Cultural Response: Cultural response on Eugonic Agar at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Brucella abortus</i> ATCC® 4315	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on bacteria and fungi from clinical specimens, refer to appropriate procedures. For the examination of bacteria and fungi in food refer to standard methods.

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Eugonic Agar is not recommended as a blood agar base for hemolytic reactions because of its high sugar content.

Packaging

Eugonic Agar	Code No.	7135A	500 g
		7135B	2 kg
		7135C	10 kg

References

1. **Vera, H. D.** 1947. The ability of peptones to support surface growth of lactobacilli. J. Bacteriol. **54**:14.
2. **MacFaddin, J. D.** 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 131-143. Williams & Wilkins, Baltimore, MD.
3. **Niven.** 1949. J. Bacteriol. **58**:633.
4. **Harrison, A. P., Jr., and P. A. Hansen.** 1950. The bacterial flora of the cecal feces of healthy turkeys. J. Bacteriol. **59**:197.
5. **Frank, H. A.** 1955. The influence of various media on spore count determinations of a putrefactive anaerobe. J. Bacteriol. **53**:561.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

FASTIDIOUS ANAEROBE AGAR (7531)

Intended Use

Fastidious Anaerobe Agar is used for the cultivation of anaerobic microorganisms.

Product Summary and Explanation

Fastidious Anaerobe Agar is a custom formulation used for the cultivation of various fastidious anaerobes from clinical and nonclinical specimens. Anaerobic bacteria are the most common organisms colonizing humans, and a frequent cause of serious infections.¹ Typically, anaerobic infections are characterized by polymicrobial mixtures of aerobic and anaerobic microbial flora, creating a challenge for anaerobic isolation.¹

Principles of the Procedure

Peptone provides nitrogen and vitamin sources in Fastidious Anaerobe Agar. Sodium Chloride maintains the osmotic balance of the medium. Soluble Starch is present to absorb any toxic metabolites. Sodium Bicarbonate increases the aerotolerance by acting as an oxygen scavenger. Sodium Pyrophosphate is a buffering agent. Glucose is the carbon source. Sodium Pyruvate is added as an energy source and as an oxygen scavenger for asaccharolytic cocci, including *Veillonella* spp. L-Cysteine HCl•H₂O is a reducing agent and growth stimulant for anaerobes. L-Arginine is added to ensure the growth of *Eubacterium lentum*,² and Hemin and Vitamin K are growth factors required by several *Bacteroides* spp.³ Sodium Succinate improves the growth of *Prevotella melaninogenica* and *Bacteroides* spp.⁴ Agar is the solidifying agent.

Formula / Liter

Peptone.....	23 g
Sodium Chloride	5 g
Soluble Starch.....	1 g
Sodium Bicarbonate.....	0.4 g
Glucose.....	1 g
Sodium Pyruvate.....	1 g
L-Cysteine HCl•H ₂ O	0.5 g
Sodium Pyrophosphate.....	0.25 g
L-Arginine.....	1 g
Sodium Succinate.....	0.5 g
Hemin.....	0.01 g
Vitamin K.....	0.001 g
Agar	12 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 45.7 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 to 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige to grey-green beige.

Prepared Appearance: Prepared medium supplemented with 5 - 10% blood is opaque and red.

Expected Cultural Response: Cultural response on Fastidious Anaerobe Agar supplemented with blood at 35°C after 48 - 72 hours of incubation under anaerobic conditions.

Microorganism	Response
<i>Bacteroides fragilis</i> ATCC® 25285	growth
<i>Clostridium perfringens</i> ATCC® 13124	growth
<i>Peptostreptococcus anaerobius</i> ATCC® 27337	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for the isolation and identification of anaerobic bacteria.

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing or appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Fastidious Anaerobe Agar	Code No.	7531A	500 g
		7531B	2 kg
		7531C	10 kg

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Sperry, J. F. and T. D. Wilkins.** 1976. Arginine, a growth-limiting factor for *Eubacterium lentum*. J. Bacteriol. **127**:780-784.
3. **Gibbons, R. J. and J. B. MacDonald.** 1960. Haemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. J. Bacteriol. **80**:164-170.
4. **Keudell, K. C. and A. F. Milford.** 1971. Succinate as a growth factor for *Bacteroides melaninogenicus*. J. Bact. **108**:175-178.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

FLUID THIOGLYCOLLATE MEDIUM (7137)

Intended Use

Fluid Thioglycollate Medium is used for sterility testing. This formula conforms to the US Pharmacopeia (USP).¹

Product Summary and Explanation

Quastel and Stephenson² found that the presence of small amounts of a compound containing an –SH group (cystein, thioglycollic acid and glutathione) permitted “aerobic” growth of *Clostridium sporogenes*. Falk, Bucca and Simmons,³ discovered the advantages of using small quantities of agar in detecting contaminants during sterility testing. The value of a small amount of agar and a reducing substance was demonstrated by Brewer.⁴

Fluid Thioglycollate Medium is also referred to as Thioglycollate Medium, and abbreviated FTM. Fluid Thioglycollate Medium is prepared according to the formula specified in the FDA Bacteriological Analytical Manual (BAM),⁵ and the AOAC Official Methods of Analysis⁶ for the examination of food, and sporicidal effects of disinfectants. FTM is recommended for sterility checks on banked blood,⁷ and blood cultures.⁸

Principles of the Procedure

Fluid Thioglycollate Medium supports the growth of a large variety of fastidious microorganisms having a wide range of growth requirements. The nitrogen, vitamin, and carbon source is provided by Enzymatic Digest of Casein and Yeast Extract. Sodium Thioglycollate and L-Cystine lower the oxidation-reduction potential of the medium by removing oxygen to maintain a low Eh. By creating an environment with a low Eh, the reducing agents prevent the accumulation of peroxides that can be toxic to some organisms. The sulfhydryl groups (-SH) of these compounds also neutralize the antibacterial effect of mercurial preservatives, making thioglycollate media useful in testing material containing heavy metals.

Resazurin is the oxidation indicator. In the oxidized state, resazurin turns pink. In the reduced state resazurin is colorless. Dextrose is included in this formula to enhance organism growth. Sodium Chloride maintains the osmotic balance of the medium. The requirement for a sealed environment is eliminated with the addition of Agar, which retards dispersion of CO₂, diffusion of oxygen, and reducing substances.⁹

Formula / Liter

Enzymatic Digest of Casein	15 g
Yeast Extract.....	5 g
Dextrose.....	5.5 g
L-Cystine	0.5 g
Sodium Chloride	2.5 g
Sodium Thioglycollate.....	0.5 g
Resazurin.....	0.001 g
Agar	0.75 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 29.8 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

NOTE: Unless used on the same day of preparation, the prepared tubes should be boiled (with caps loose) for 3 - 5 minutes and cooled before use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy, and light amber in color with a pink upper layer. If the pink layer is greater than 10% of the tube, the medium may be restored once by heating in a steam bath until the pink color disappears.

Expected Cultural Response: Cultural response at an inoculum level of 1 – 100 cfu's at 35°C after 2 - 7 days incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 6633	growth
<i>Bacteroides vulgatus</i> ATCC® 8482	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Micrococcus luteus</i> ATCC® 9341	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Fluid Thioglycollate Medium.

Results

Typically growth is visually observed in the medium. Gram-negative bacilli usually grow diffusely, Gram-positive cocci exhibit puff-ball type growth, and strict aerobes, such as pseudomonads and yeast, tend to grow in a thin layer on the surface of the broth.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Fluid Thioglycollate Medium	Code No.	7137A	500 g
		7137B	2 kg
		7137C	10 kg

References

1. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.
2. **Quastel and Stephenson.** 1926. General biological products standards. Fed. Regist. **21**:6109.12.
3. **Falk, C. R., H. Bucca, and M. P. Simmons.** 1939. A comparative study of the use of varying concentrations of agar in the the medium used to detect contaminants in biological products, J. Bacteriol. **37**:121-131.
4. **Brewer, J. H.** 1940. Clear liquid mediums for the "aerobic" cultivation of anaerobes. J. Amer. Med. Assoc. **115**:598-600.
5. **Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Association of Official Analytical Chemists.** 1995. Official Methods of Analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
7. **Federal Register.** 1992. Additional standard for human blood and blood products. Fed Regist. **21**:640.2.17.
8. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D.C.
9. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification maintenance of medical bacteria, vol. 1, p. 755-762. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

FRASER BROTH (7626)

Intended Use

Fraser Broth is used with acriflavin, nalidixic acid and ferric ammonium citrate for the selective enrichment of *Listeria* spp.

Product Summary and Explanation

Listeria monocytogenes, described first in 1926 by Murray, Webb and Swann, is an extensive problem in public health and food industries.¹ This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.² Epidemiological evidence from outbreaks of listeriosis indicated the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey frankfurters, coleslaw, pasteurized milk, Mexican style cheese and pate'.⁴ *Listeria* spp. are ubiquitous in nature, present in a wide range of unprocessed foods and in soil, sewage, and river water.⁵

Fraser Broth is based on the formulation of Fraser and Sperber.⁶ This medium is used in rapid detection of *Listeria* from food⁷ and environmental samples. *Listeria* spp. grow over a pH range of 5.0 - 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, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* spp. is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract provide nitrogen, vitamins and minerals in Fraser Broth. The Phosphates are the buffering agents, Sodium Chloride maintains osmotic balance. 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. Blackening of the medium by esculin-hydrolyzing bacteria is a result of formation of 6,7-dihydroxycoumarin that reacts with ferric ions.⁶ Selectivity is provided by the presence of Lithium Chloride, Nalidixic Acid and Acriflavin in the formula. The high salt tolerance of *Listeria* spp. is used to inhibit growth of enterococci.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Beef Extract	5 g
Yeast Extract.....	5 g
Sodium Chloride	20 g
Disodium Phosphate.....	12 g
Monopotassium Phosphate	1.35 g
Esculin	1 g
Lithium Chloride	3 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / 10 mL

Ferric Ammonium Citrate, 0.5 g
Nalidixic Acid, 20 mg
Acriflavin, 25 mg

Precautions

1. For Laboratory Use.
2. TOXIC. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 57.4 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool broth to room temperature.
4. Aseptically add 10 mL of a filter sterilized solution containing 0.5 g ferric ammonium citrate, 20 mg nalidixic acid and 25 mg acriflavin.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and tan.

Prepared Appearance: Prepared medium is medium amber, clear to slightly opalescent with a fine precipitate.

Expected Cultural Response: Cultural response in Fraser Broth at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	growth w/blackening
<i>Listeria monocytogenes</i> ATCC® 15313	growth w/blackening
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited (@ 18-24 hrs)

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

To isolate *Listeria monocytogenes* from processed meats and poultry, the following procedure is recommended by the U.S.D.A.⁷

1. Add 25 g of test material to 225 mL of UVM Modified Listeria Enrichment Broth and mix or blend thoroughly. Incubate for 20 - 24 hours at 30°C.
2. Transfer 0.1 mL of the incubate broth to Fraser Broth. Incubate at 35°C for 26 ± 2 hours.
3. At 24 and 48 hours, streak the Fraser Broth culture to Modified Oxford Agar. Incubate the Modified Oxford plates at 35°C for 24 - 48 hours.

Results

1. Examine agar plates for suspect colonies. For complete identification and confirmation of *Listeria* spp., consult appropriate references.⁷⁻¹⁰
2. Rapid slide and macroscopic tube tests can be used for definitive serological identification.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

An identification of *Listeria monocytogenes* must be confirmed by biochemical and serological testing.^{9,10}

Packaging

Fraser Broth	Code No.	7626A	500 g
		7626B	2 kg
		7626C	10 kg

References

1. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bact. **29**:407-439.
2. Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen refrigerated ground beef. J. Food Prot. **57**:969-974.
3. Bremer, P. J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
4. Grau, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
5. Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
6. Fraser, J., and W. Sperber. 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. **51**:762-765.
7. Lee, W. H., and D. McClain. 1994. Laboratory Communication No. 57, U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
8. Vanderzant, C., and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
9. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
10. Marshall, R. T. (ed.). Standard methods for the examination of dairy products 16th ed., American Public Health Association, Washington D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

FRASER BROTH BASE (7502)

Intended Use

Fraser Broth Base is used with ferric ammonium citrate for the selective enrichment of *Listeria* species.

Product Summary and Explanation

Listeria monocytogenes, described in 1926 by Murray, Webb and Swann, is a widespread problem in public health and food industries.¹ This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and susceptible pregnant women.² Epidemiological evidence from outbreaks of listeriosis indicate the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey frankfurters, coleslaw, pasteurized milk, Mexican style cheese and pate'.⁴ *Listeria* species are ubiquitous in nature, present in a wide range of unprocessed foods and in soil, sewage, and river waste.⁵

Fraser Broth Base is based on the formulation of Fraser and Sperber.⁶ This medium is used in rapid detection of *Listeria* from food⁷ and environmental samples. *Listeria* species grow over a pH range of 5.0 - 9.6, and can survive in food products with pH levels outside these parameters.⁸ *Listeria* species are microaerophilic, Gram-positive, asporogenous, non-encapsulated, non-branching, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract provide nitrogen, vitamins, and minerals in Fraser Broth Base. The Phosphates are the buffering agents. Sodium Chloride maintains osmotic balance. 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 formation of 6,7-dihydroxycoumarin that reacts with ferric ions.⁶ Selectivity is provided by the presence of Lithium Chloride, Nalidixic Acid, and Acriflavin in the formula. The high salt tolerance of *Listeria* is used to inhibit growth of enterococci.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Beef Extract	5 g
Yeast Extract.....	5 g
Sodium Chloride	20 g
Disodium Phosphate.....	9.6 g
Monopotassium Phosphate	1.35 g
Esculin	1 g
Acriflavin	0.024 g
Nalidixic Acid.....	0.020 g
Lithium Chloride	3 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / 10 mL

5% Ferric Ammonium Citrate, 10 mL
filtered sterilized aqueous solution /L

Precautions

1. For Laboratory Use.
2. TOXIC. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 55 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Aseptically add 10 mL of a filtered sterilized 5% aqueous solution of ferric ammonium citrate.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is golden yellow with an opalescent top and clear to slight hazy.

Expected Cultural Response: Cultural response in Fraser Broth Base at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	growth w/blackening
<i>Listeria monocytogenes</i> ATCC® 15313	growth w/blackening
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited (@ 18-24 hrs)

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

To isolate *Listeria monocytogenes*, the following procedure is recommended by U.S.D.A.⁷

1. Add 25 g of test material to 225 mL of UVM Modified Listeria Enrichment Broth and mix or blend thoroughly. Incubate for 20 - 24 hours at 30°C.
2. Transfer 0.1 mL of the incubated broth to Fraser Broth. Incubate at 35°C for 26 ± 2 hours.
3. At 24 and 48 hours, streak the Fraser Broth culture to Modified Oxford Agar. Incubate the Modified Oxford plates at 35°C for 24 - 48 hours.

Results

For further identification and confirmation of *Listeria* species, consult appropriate references.^{7,8,9,10} Rapid slide and macroscopic tube tests can be used for definitive serological identification.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Fraser Broth Base	Code No.	7502A	500 g
		7502B	2 kg
		7502C	10 kg

References

1. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bacteriol. **29**:407-439.
2. Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. Bremer, P. J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
4. Grau, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
5. Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
6. Fraser, J., and W. Sperber. 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. **51**: 762-765.
7. Lee, W. H., and D. McClain. 1994. Laboratory Communication No. 57, U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
8. Vanderzant, C., and D. F. Splittstoesser (eds). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
9. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
10. Marshall, R. T. (ed.)., Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

FUNGISEL AGAR (7205)

Intended Use

Fungisel Agar is used for the selective isolation of pathogenic fungi from clinical materials.

Product Summary and Explanation

Fungisel Agar contains Cycloheximide and Chloramphenicol. These antibiotics selectively inhibit saprophytic fungi and bacteria while allowing pathogenic fungi to grow. Adding antimicrobial agents to media for the isolation of pathogenic fungi is well documented.^{1,2,3}

Selective fungal media are recommended for the isolation of dermatophytes, because these pathogens are not sensitive to Cycloheximide or Chloramphenicol.⁴

Principles of the Procedure

Enzymatic Digest of Soybean Meal provides the nitrogen and vitamin source required for organism growth in Fungisel Agar. Dextrose is included as an energy source. Cycloheximide and Chloramphenicol are used to restrict the growth of bacteria and commensal yeast. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Soybean Meal 10 g
Dextrose..... 40 g
Cycloheximide..... 0.4 g
Chloramphenicol..... 0.05 g
Agar 15.5 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. VERY TOXIC. Toxic by inhalation and if swallowed. Possible risk of irreversible effects. Possible risk of harm to the unborn child.

Directions

1. Suspend 36 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium is clear to trace hazy, and light to medium yellow.

Expected Cultural Response: Cultural response on Fungisel Agar at 30°C after 2 – 7 days of incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	partial to complete inhibition
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi. Primary isolation should include the use of both non-selective and selective media.³
3. Antibiotic-containing media should be incubated at room temperature. Additional procedures may be required for complete identification of pathogenic fungi and yeasts.

Packaging

Fungisel Agar	Code No.	7205A	500 g
		7205B	2 kg
		7205C	10 kg

References

1. **Georg, L. K., L. Ajello, and C. Papageorge.** 1954. Use of cycloheximide in the selective isolation of fungi pathogenic to man. J. Lab Clin. Med., **44**:422-428.
2. **Murray, P.R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, MD.
4. **Georg, L. K., L. Ajello, E. S. McDonough, and S. Brinkman.** 1960. In vitro effects of antibiotics on yeast phase of *Blastomyces dermatitidis* and other fungi. J. Lab & Clin. Med. **55**:116-119.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

GC AGAR (7104)

Intended Use

GC Agar is used with hemoglobin and enrichment for the isolation and cultivation of *Neisseria gonorrhoeae* and other fastidious organisms.

Product Summary and Explanation

In 1945, Johnston described a medium that successfully grew colonies of *N. gonorrhoeae* in 24 rather than 48 hours.¹ GC Agar was introduced in 1947 with reduced agar content. While investigating the growth rate of gonococcal strains, a medium containing growth factors glutamine and cocarboxylase was found to improve recovery.² In 1964, Thayer and Martin formulated a selective medium incorporating the antibiotics Polymyxin B and Rostocetin, with added supplements, into GC Agar.³ Thayer and Martin improved their medium by replacing the original antibiotics with a new microbial solution of Colistin, Vancomycin, and Nystatin (CVN).⁴ In 1970, Martin and Lester improved the new Thayer-Martin Medium by increasing the agar and glucose content and adding a new antibiotic, Trimethoprim Lactate (T).⁵ This improved medium was called Modified Thayer-Martin (MTM) Medium. Martin and Lewis improved selectivity of MTM by increasing the concentration of Vancomycin and replacing Nystatin with Anisomycin for greater inhibition of yeasts, known as Martin Lewis (ML) Agar.⁶ Transgrow Medium is a transport medium system incorporating either MTM or ML formulations.⁷

Principles of the Procedure

GC Agar is employed as a basal medium in the preparation of Chocolate Agar, Thayer-Martin Medium, Modified Thayer-Martin Medium, Martin-Lewis Agar, and Transgrow Agar.

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and minerals in GC Agar. Corn Starch absorbs any toxic metabolites produced. The Phosphates are buffering agents. Sodium Chloride maintains osmotic balance of the medium. Agar is the solidifying agent. Chocolate Agar is prepared from GC Agar with the addition of 2% Hemoglobin. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* and enhanced growth of *Neisseria* spp. A chemical enrichment composed of cofactors, vitamins, and nicotinamide adenine dinucleotide (NAD) are also required for growth of *Haemophilus* and *Neisseria* spp. If required, antimicrobial supplements are added as inhibitors for improved selectivity of the medium.

Formula / Liter

Enzymatic Digest of Casein	7.5 g
Enzymatic Digest of Animal Tissue.....	7.5 g
Corn Starch.....	1 g
Dipotassium Phosphate.....	4 g
Monopotassium Phosphate	1 g
Sodium Chloride	5 g
Agar	10 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Hemoglobin Solution, 2%, 100 mL
Growth Enrichment, 2 mL
Antimicrobials, if required

Precaution

1. For In Vitro Diagnostic Use.

Directions, Double Strength

1. Suspend 7.2 g of the medium in 100 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 – 50°C.
4. Prepare 100 mL of a 2% hemoglobin solution and autoclave at 121°C for 15 minutes.
5. Cool to 45 - 50°C and aseptically add to the molten GC Agar. Add 2 mL of growth enrichment. Add antimicrobials, if desired. Mix thoroughly and dispense.

Note: Refer to appropriate references for media formulations of Thayer-Martin Medium, Modified Thayer-Martin Medium, Martin Lewis Agar, and Transgrow Agar.^{8,9}

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared GC Agar supplemented as Chocolate Agar is opaque and brown.

Expected Cultural Response: Cultural response on Chocolate Agar at 35°C under CO₂ enrichment after 18 – 24 hours incubation.

Microorganism	Response
<i>Haemophilus influenza</i> ATCC® 10211	growth
<i>Neisseria gonorrhoeae</i> ATCC® 43070	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus agalactiae</i> ATCC® 13813	growth
<i>Streptococcus pneumoniae</i> ATCC® 6303	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of *Neisseria* spp. and *Haemophilus* spp. consult procedures outlined in the references.^{8,9}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Although certain diagnostic tests may be performed directly on GC Agar, biochemical and immunological testing using pure cultures are recommended for complete identification.

Packaging

GC Agar	Code No.	7104A	500 g
		7104B	2 kg
		7104C	10 kg

References

1. Johnson, J. 1945. Comparison of gonococcus cultures read at 24 and 48 hours. J. Vener. Dis. Inform. **26**:239.
2. Lankford, C. E., V. Scott, M. F. Cox, and W. R. Cooke. 1943. Some aspects of nutritional variation of the gonococcus. J. Bacteriol. **45**:321.
3. Thayer, J. D., and J. E. Martin, Jr. 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. Public Health Rep. **81**:559.
4. Thayer, J. D., and A. Lester. 1971. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. HSMHA Health Service Rep. **86**:30.
5. Martin, J. E., Jr., and R. L. Jackson. 1975. A biological environmental chamber for the culture of *N. gonorrhoeae* with a new commercial medium. Public Health Rep. **82**:361.
6. Martin, J. E., Jr., and J. S. Lewis. 1977. Anisomycin: improved anti-mycotic activity in modified Thayer-Martin Medium. Public Health Rep. **35**:53.
7. MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, MD.
8. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook. vol. 1. American Society for Microbiology, Washington, D.C.
9. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

GELATIN (7202)

Intended Use

Gelatin is a protein source and solidifying agent for use in preparing microbiological culture media.

Product 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, tendons, cartilage and similar animal tissues.¹ Koch 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 investigating bacterial growth.¹ 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. Several media containing Gelatin are specified in standard methods for multiple applications.^{2,3}

Principles of the Procedure

The melting point of a 12% concentration of gelatin is between 28 and 30°C, allowing it to be used as a solidifying agent. Certain microorganisms elaborate gelatinolytic enzymes (gelatinases) which hydrolyze gelatin, causing liquefaction of a solidified medium. Gelatin is also used as a nitrogen and amino acid source.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is granular, homogeneous, free-flowing and light beige.

Prepared Appearance (2% wt/vol): Prepared medium is light amber, clear to slightly opalescent, with no or a light precipitate.

pH (2% Solution at 25°C): 4.5 - 5.5

Expected Cultural Response: Cultural response in Staphylococcus Agar #110 after incubation at 35°C for 18 - 48 hours.

Microorganism	Response
<i>Staphylococcus aureus</i> ATCC 25923	good to excellent growth mannitol and gelatinase positive
<i>Staphylococcus epidermidis</i> ATCC 12228	good to excellent growth mannitol and gelatinase negative

Test Procedure

Refer to appropriate references for specific procedures using Gelatin.

Results

Refer to appropriate references for test results.

Storage

Store sealed container of Gelatin at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Gelatin should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Gelatin in its intact container when stored as directed.

Packaging

Gelatin	Code No.	7202A	500 g
		7202B	2 kg
		7202C	10 kg

References

1. **Gershenfeld, L., and L. F. Tice.** 1941. Gelatin for bacteriological use. J. Bacteriol. **41**:645-652.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed., American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

GN BROTH (Hajna) (7218)

Intended Use

GN Broth (Hajna) is used for the selective enrichment of Gram-negative organisms.

Product Summary and Explanation

Hajna formulated Gram Negative (GN) Broth as an enrichment medium for enteric gram-negative bacilli, especially *Salmonella* spp. and *Shigella* spp.^{1,2,3} Croft and Miller demonstrated improved recovery of *Shigella* spp. using GN Broth enrichment compared to direct inoculation of agar.⁴ Taylor and Schelhart reported improved recovery of *Salmonella* spp. and *Shigella* spp. when using GN Broth enrichment compared to direct inoculation of media.⁵ Taylor and Schelhart found GN Broth to be superior to selenite enrichment medium for recovering *Shigella* spp.⁶

GN Broth, Hajna, is recommended as an enteric enrichment broth for clinical specimens.^{7,8} This broth is used as a nonselective enrichment to recover *Salmonella* spp. and *Shigella* spp. from food.⁹

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are used as a nitrogen and vitamin source in this medium. Dextrose and Mannitol are the fermentable carbohydrates. The higher concentration of Mannitol over Dextrose favors growth of mannitol-fermenting *Salmonella* spp. and *Shigella* spp. over mannitol non-fermenting species, such as *Proteus*. The Phosphates are buffering agents. Sodium Citrate and Sodium Deoxycholate inhibit growth of gram-positive bacteria and coliforms. Sodium Chloride maintains the osmotic balance of the medium.

Formula/Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Dextrose.....	1 g
Mannitol	2 g
Sodium Citrate	5 g
Sodium Deoxycholate	0.5 g
Dipotassium Phosphate.....	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 39 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is gold to light amber and clear.

Expected Cultural Response: Cultural response after 24 - 48 hours incubation at 35°C.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Enterococcus faecalis</i> ATCC® 29212	partial to complete inhibition
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Shigella sonnei</i> ATCC® 25931	growth

The organisms listed are the minimum that should be performed for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

Growth of gram-negative organisms, especially *Salmonella* spp. and *Shigella* spp. is enhanced.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

GN Broth (Hajna)	Code No.	7218A	500 g
		7218B	2 kg
		7218C	10 kg

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 357-359. Williams & Wilkins, Baltimore, MD.
2. **Hajna, A. A.** 1955. A new specimen preservative for gram-negative organisms of the intestinal group. Public Health Lab. **13**:59-62.
3. **Hajna, A. A.** 1955. A new enrichment broth medium for gram-negative organisms of the intestinal group. Public Health Lab. **13**:83-89.
4. **Croft, C. C., and M. J. Miller.** 1956. Isolation of *Shigella* from rectal swabs with Hajna "GN" broth. Am. J. Clin. Path. **26**:411-417.
5. **Taylor, W. I., and D. Schelhart.** 1967. Isolation of shigellae, IV. Comparison of plating media with stools. Am. J. Clin. Path. **48**:356-362.
6. **Taylor, W. I., and D. Schelhart.** 1968. Isolation of shigellae, V. Comparison of enrichment broths with stools. Appl. Microbiol. **16**:1383-1386.
7. **Forbes, B. A., and P. A. Granato.** 1995. Processing specimens for bacteria, p. 265-267. In P. R. Murray, E. J. Baron, M. A. Tenover, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, 1.10.8. American Society for Microbiology, Washington, D.C.
9. **Vanderzant, C. and D.F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

HC AGAR BASE (7520)

Intended Use

HC Agar Base is used with Polysorbate 80 for the enumeration of molds in cosmetics.

Product Summary and Explanation

Methods for isolating molds from cosmetic products require incubation for 5 – 7 days using traditional agar media.¹ In 1986, Mead and O'Neill described a new medium, HC Agar, for enumerating molds in cosmetic products.² The formulation of HC Agar decreased incubation time to 3 days at $27.5 \pm 0.5^\circ\text{C}$ for molds.² HC Agar Base, based on the HC Agar formula of Mead and O'Neill, is supplemented with Polysorbate 80 to prepare HC Agar.²

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, amino acids, and carbon in HC Agar Base. Yeast Extract is a vitamin source required for organism growth. Dextrose is the fermentable carbohydrate. The Phosphates buffer the pH to near neutrality. Ammonium Chloride and Magnesium Sulfate provide essential ions. Sodium Carbonate inactivates low levels of preservatives that are active at an acidic pH. Chloramphenicol inhibits bacteria, including *Pseudomonas aeruginosa* and *Serratia marcescens*, potential contaminants of cosmetic products. Polysorbate 80 neutralizes preservatives and sequesters surfactants that may be present in residual amounts from product samples.² Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Yeast Extract.....	5 g
Dextrose.....	20 g
Disodium Phosphate.....	3.5 g
Monopotassium Phosphate	3.4 g
Ammonium Chloride	1.4 g
Sodium Carbonate	1 g
Magnesium Sulfate	0.06 g
Chloramphenicol	0.1 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. VERY TOXIC. Toxic by inhalation and contact with skin.

Directions

1. Suspend 54.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Add 20 mL of Polysorbate 80 and mix.
4. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and medium to dark amber.

Expected Cultural Response: Cultural response on HC Agar Base supplemented with Polysorbate 80 at $27.5 \pm 0.5^{\circ}\text{C}$ after 2 - 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Bacillus subtilis</i> ATCC® 9372	inhibited
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate and inoculate directly onto surface of the medium.¹ Inoculate duplicate plates.
2. Incubate plates aerobically at $27.5 \pm 0.5^{\circ}\text{C}$.
3. Examine plates for growth and recovery after 72 hours incubation.
4. Count mold colonies from duplicate plates and record average count as mold count per gram or milliliter of sample.

Results

Mold colonies should yield good growth and recovery. Bacteria should be inhibited.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C . Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. The $27.5 \pm 0.5^{\circ}\text{C}$ incubation temperature is critical for obtaining statistically significant mold counts after three days.

Packaging

HC Agar Base	Code No.	7520A	500 g
		7520B	2 kg
		7520C	10 kg

References

1. Hitchins, A. D., T. T. Tran, and J. E. McCarron. 1995. Microbiological methods in cosmetics. *In* Bacteriological analytical manual, 8th ed AOAC International, Gaithersburg, MD.
2. Mead, C., and J. O'Neill. 1986. A three-day mold assay for cosmetics and toiletries. *J. Soc. Cosmet. Chem.* **37**:49-57.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

HEART INFUSION AGAR (7269)

Intended Use

Heart Infusion Agar is used for the cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Heart Infusion Agar is a non-selective general purpose medium. Heart Infusion Agar is prepared from an infusion of beef heart. The first media used for the cultivation of bacteria was prepared from meat infusions. Huntoon¹ found that pathogenic microorganisms could be grown on infusion medium without enrichments. This infusion medium can be used for mass culture of microorganisms for vaccine production. Pathogenic microorganisms, including meningococci and pneumococci, will grow on Heart Infusion Agar without enrichments.¹

Heart Infusion Agar can be used as a base for the preparation of blood agar in determining hemolytic reactions, and specified for the isolation of *Vibrio cholerae* and *Vibrio* spp.^{2,3} Several modifications of Heart Infusion media have been described.⁴

Principles of the Procedure

Beef Heart Infusion Solids, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue provides the nitrogen, vitamins, and carbon in Heart Infusion Agar. Yeast Extract provides additional vitamins to enhance organism growth. Sodium Chloride maintains osmotic balance in the medium. Agar is the solidifying agent. The addition of 5% sheep blood provides additional growth factors and is used to determine hemolytic reactions.

Formula / Liter

Beef Heart Infusion Solids	2 g
Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	5 g
Yeast Extract.....	2 g
Sodium Chloride	5 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 to 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear, and light to medium amber.

Expected Cultural Response: Cultural response on Heart Infusion Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Neisseria meningitidis</i> ATCC® 13090	growth	---
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Heart Infusion Agar.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Heart Infusion Agar	Code No.	7269A	500 g
		7269B	2 kg
		7269C	10 kg

References

1. **Huntoon, F. M.** 1918. "Hormone" Medium. A simple medium employable as a substitute for serum medium. J. of Infect. Dis. **23**:169-172.
2. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8thed., AOAC International, Gaithersburg, MD.
3. **Vanderzant, C. and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 426-431. CRC Press, Boca Raton, FL.

Technical Information

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HEART INFUSION BROTH (7319)

Intended Use

Heart Infusion Broth is used for the cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Heart Infusion Broth is a non-selective general purpose medium. Heart Infusion Broth is prepared from an infusion of beef heart. The first media used for the cultivation of bacteria was prepared from meat infusions. Huntoon¹ found that pathogenic microorganisms could be grown on infusion medium without enrichments. This infusion medium can be used for mass culture of microorganisms for vaccine production. Pathogenic microorganisms, including meningococci and pneumococci, will grow in Heart Infusion Broth.¹

Heart Infusion Broth can be used as the base in carbohydrate fermentation tests.² Several modifications of Heart Infusion media have been described.³

Principles of the Procedure

Beef Heart Infusion Solids, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue provides the nitrogen, vitamins, and carbon in Heart Infusion Broth. Yeast Extract provides additional vitamins to enhance organism growth. Sodium Chloride maintains osmotic balance in the medium.

Formula / Liter

Beef Heart Infusion Solids	2 g
Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	6 g
Yeast Extract.....	2 g
Sodium Chloride	5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 25 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and beige.

Prepared Appearance: Prepared medium is clear, and gold to amber.

Expected Cultural Response: Cultural response in Heart Infusion Broth at 35°C after 24 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Heart Infusion Broth.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Heart Infusion Broth	Code No.	7319A	500 g
		7319B	2 kg
		7319C	10 kg

References

1. **Huntoon, F. M.** 1918. "Hormone" Medium. A simple medium employable as a substitute for serum medium. J. of Infect. Dis. **23**:169-172.
2. **Ruoff, K. L.** 1995. *Streptococcus*, p. 305. In Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, Washington, D.C.
3. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 426-431. CRC Press, Boca Raton, FL.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

HEKTOEN ENTERIC AGAR (7138)

Intended Use

Hektoen Enteric Agar is used for the isolation and differentiation of enteric pathogens.

Product Summary and Explanation

Hektoen Enteric Agar was developed in 1967 by King and Metzger.^{1,2} Compared to other enteric differentiating media commonly used in clinical laboratories, Hektoen Enteric Agar increased the isolation rate of *Salmonella* spp. and *Shigella* spp. This was accomplished by increasing the carbohydrate and peptone content of the medium in order to counteract the inhibitory effects of bile salts and indicators. King and Metzger formulated a medium that slightly inhibited growth of *Salmonella* and *Shigella*, while inhibiting gram-positive microorganisms.^{1,2}

Hektoen Enteric Agar is used to isolate and differentiate *Salmonella* spp. and *Shigella* spp., both of which cause a variety of serious human gastrointestinal illnesses.³ Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and or neurological damage.³ This infection can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella* spp. U.S. federal guidelines require various poultry products to be routinely monitored before distribution for human consumption. A variety of procedures have been developed using Hektoen Enteric Agar as part of the multi-step procedure to isolate *Salmonella* spp. from food samples.⁴⁻⁷

Principles of the Procedure

Enzymatic Digest of Animal Tissue provides nitrogen, carbon, and amino acids required for organism growth. Yeast Extract is a vitamin source. Bile Salts Mixture and Acid Fuchsin inhibit gram-positive organisms. Lactose, Sucrose, and Salicin are fermentable carbohydrates. Sodium Chloride maintains the osmotic balance of the medium. Ferric Ammonium Citrate, a source of iron, allows production of hydrogen sulfide (H₂S) present from Sodium Thiosulfate. H₂S-positive colonies have black centers. Bromothymol Blue is added as the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	16.5 g
Yeast Extract.....	3 g
Bile Salts Mixture	4.5 g
Lactose	12 g
Sucrose.....	12 g
Salicin	2 g
Sodium Chloride	5 g
Sodium Thiosulfate	5 g
Ferric Ammonium Citrate.....	1.5 g
Bromthymol Blue.....	0.065 g
Acid Fuchsin	0.1 g
Agar	13.5 g

Final pH: 7.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. **HARMFUL.** May be harmful if swallowed or inhaled. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 75 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and light to dark green.

Expected Cultural Response: Cultural response on Hektoen Enteric Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	yellow to salmon colonies
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	---
<i>Shigella flexneri</i> ATCC® 12022	growth	green colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	green to green-blue colonies with black centers

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For isolation and identification of pathogenic *Enterobacteriaceae* refer to appropriate references.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Do not autoclave medium because excessive heat may alter ingredients.
2. *Proteus* spp. may resemble salmonellae or shigellae. Further testing should be conducted to confirm the presumptive identification or organisms isolated on this medium.
3. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Hektoen Enteric Agar	Code No.	7138A	500 g
		7138B	2 kg
		7138C	10 kg

References

1. **King, S., and W. I. Metzger.** 1968. A new plating medium for the isolation of enteric pathogens. Appl. Microbiol. **16**:577-578.
2. **King, S., and W. I. Metzger.** 1968. A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with S and EMB Agar. Appl Microbiol. **16**:579-581.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytic Chemists.** 1996. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.
6. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** FDA Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Flowers, R. S., W. H. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products. In Marshall, R. T. (ed.). Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

HEMOGLOBIN POWDER (7195)

Intended Use

Hemoglobin Powder is freeze dried hemoglobin for use in preparing microbiological culture media.

Product Summary and Explanation

Hemoglobin Powder is obtained from desiccated beef blood. Hemoglobin Powder is used with GC Agar in the preparation of Chocolate Agar, Thayer-Martin Medium, Modified Thayer-Martin Medium, Martin-Lewis Agar, and Transgrow Agar. Supplemented with Hemoglobin Powder and growth enrichment, these enriched media are used for the isolation and cultivation of fastidious microorganisms, especially *Neisseria* spp. and *Haemophilus* spp.

Principles of the Procedure

Hemoglobin Powder provides hemin (X factor) required for growth of *Haemophilus* and for enhanced growth of *Neisseria* spp. In the preparation of Chocolate Agar, Hemoglobin Powder is used in a 2% solution.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and dark brown in color.

Prepared Appearance (2.0% wt/vol): Prepared medium is opaque and light brown.

pH (2% Solution at 25°C): 7.8 - 8.6

Expected Cultural Response: Cultural response in Chocolate Agar after incubation at 35°C for 18 - 24 hours under increased CO₂.

Microorganism	Response
<i>Neisseria meningitidis</i> ATCC 13090	good growth
<i>Haemophilus Influenzae</i> ATCC 10211	good growth

Test Procedure

Refer to appropriate references for specific procedures using Hemoglobin Powder. For a complete discussion on the isolation and identification of *Neisseria* spp. and *Haemophilus* spp. refer to procedures outlined in the references.^{1,2}

Results

Refer to appropriate references for test results.

Storage

Store sealed container of Hemoglobin Powder at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Hemoglobin Powder should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to Hemoglobin Powder in its intact container when stored as directed.

Packaging**Hemoglobin Powder****Code No.****7195A****500 g****7195B****2 kg****7195C****10 kg****References**

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. Vol. 1. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

INHIBITORY MOLD AGAR (7238)

Intended Use

Inhibitory Mold Agar is used for the selective isolation of pathogenic fungi.

Product Summary and Explanation

Inhibitory Mold Agar, containing Chloramphenicol, is a moderately selective medium formulated by Ulrich.¹ This medium can be used as a general cultivation medium for various strains of pathogenic fungi, especially *Hisptoplasma capsulatum* and dermatophytes. Chloramphenicol selectively inhibits saprophytic fungi and bacteria while allowing pathogenic fungi to grow. Adding antimicrobial agents to media for the isolation of pathogenic fungi is documented.^{2,3,4} Selective fungal media are recommended for the isolation of dermatophytes because these pathogens are not sensitive to Chloramphenicol.⁵

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and amino acids in Inhibitory Mold Agar. Yeast Extract is the vitamin source. Dextrose, Starch, and Dextrin are energy sources for the metabolism of fungi. Sodium Chloride maintains the osmotic balance of the medium. Sodium Phosphate is a buffering agent. Magnesium Sulfate, Ferrous Sulfate, and Manganese Sulfate provide essential ions and minerals. Chloramphenicol is a broad-spectrum antibiotic that inhibits a wide range of gram-positive and gram-negative bacteria. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	3 g
Enzymatic Digest of Animal Tissue.....	2 g
Yeast Extract.....	5 g
Dextrose.....	5 g
Starch.....	2 g
Dextrin.....	1 g
Sodium Phosphate.....	2 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Sodium Chloride	0.04 g
Manganese Sulfate	0.16 g
Chloramphenicol.....	0.125 g
Agar	15 g

Final pH: 6.7 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. VERY TOXIC. Toxic by inhalation and contact with skin.

Directions

1. Suspend 36 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 118 - 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to trace hazy and light yellow beige.

Expected Cultural Response: Cultural response on Inhibitory Mold Agar at 25 - 30°C up to 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for procedures on the isolation of pathogenic fungi.

Results

After sufficient incubation, plates should reveal isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Examine plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi. Primary isolation should include use of both non-selective and selective media.⁴

Packaging

Inhibitory Mold Agar	Code No.	7238A	500 g
		7238B	2 kg
		7238C	10 kg

References

1. **Ulrich, J. A.** 1956. Media and methods for the isolation and identification of pathogenic fungi. Bacteriol. Proc. SAB, M75, p. 87.
2. **Georg, L. K., L. Ajello, and C. Papageorge.** 1954. Use of cycloheximide in the selective isolation of fungi pathogenic to man. J. Lab Clin. Med. **44**:422-428.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
5. **Georg, L. K., L. Ajello, E. S. McDonough, and S. Brinkman.** 1960. In vitro effects of antibiotics on yeast phase of *Blastomyces dermatitidis* and other fungi. J. Lab & Clin. Med. **55**:116-119.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

BMA AGAR (6903)

Intended Use

BMA (Buffered Mug Agar) is used with LMG Agar and the ISO-GRID Membrane Filtration System for the direct enumeration of Glucuronidase-Positive *Escherichia coli*.

Product Summary and Explanation

Buffered MUG Agar is formulated to take advantage of the ability of 4-methylumbelliferone to fluoresce at an alkaline pH under long wave (365 nm) ultraviolet light. The formula consists of phosphate buffered saline modified to contain 4-methylumbelliferyl- β -D-glucuronide (100 mg/L) and 1.5% (w/v) agar. The formula enables rapid detection of β -glucuronidase activity in bacterial colonies previously exposed to sodium glucuronate, an inducer of β -glucuronidase production by *E. coli*.

Buffered MUG Agar is recommended to be used with Lactose Monensin Glucuronate (LMG) Agar for detection and enumeration of *E. coli* from all foods using the ISO-GRID Filtration Method.^{1,2}

Principles of the Procedure

Sodium Phosphate, Monobasic and Dibasic, buffer the final pH of the medium to 7.4 ± 0.2 . Sodium Chloride maintains the osmotic environment. 4-Methylumbelliferyl- β -D-Glucuronide (MUG) is a fluorogenic substrate for β -glucuronidase. *E. coli* β -glucuronidase attacks MUG to yield 4-methylumbelliferone, a water soluble compound producing a blue-white fluorescence under long wave (365 nm) ultraviolet light. Agar is the solidifying agent.

Formula / Liter

Sodium Phosphate, Dibasic..... 8.23 g
Sodium Phosphate, Monobasic 1.2 g
Sodium Chloride 5 g
4-Methylumbelliferyl- β -D-Glucuronide 0.1 g
Agar 15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. Irritant. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 29.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light to medium tan.

Prepared Appearance: Prepared medium is clear to trace hazy and very light to light beige.

Expected Cultural Response: Cultural response of quality control organisms were inoculated on LMG Agar using the ISO-GRID Filtration System method. After incubation, the filters were transferred to Buffered MUG Agar, incubated at $35 \pm 1.0^{\circ}\text{C}$ for 2 - 5 hours and examined under long wave (366 nm) ultraviolet light.

Microorganism	Response	Reaction
<i>Escherichia coli</i> ATCC 25922	good growth	blue-white fluorescence
<i>Citrobacter freundii</i> ATCC 8090	good growth	no fluorescence

Test Procedure

1. After obtaining the Total Coliform Count on LMG Agar, use sterile forceps to transfer the ISO-GRID membrane filter, grid side up, to a BMA plate.
2. Incubate the BMA plate for 2 - 5 hours at 35 - 37°C.
3. Examine the membrane filter under long wave ultraviolet light.

Results

E. coli colonies fluoresce bright blue-white. Ignore any colonies with a pale yellow fluorescence.

If positive colonies are present, count the number of squares containing *E. coli*. Convert the number of squares to the corresponding MPN and calculate the confirmed *E. coli* MPN, using the methods described in the ISO-GRID Methods Manual.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Approximately 6% of the *E. coli* strains including the enterohemorrhagic (O157:H7) strains are glucuronidase negative and will be missed on this medium.³ Alternate procedures must be used for these organisms. Please consult appropriate references.⁴

Packaging

BMA Agar (Buffered MUG Agar)

Code No.

6903A

500 g

References

1. **Entis, P.** 1989. Hydrophobic grid membrane filter/MUG method for total coliform and *Escherichia coli* enumeration in foods: Collaborative study. J. AOAC. **72**:936-950.
2. **Entis, P., and P. Boleszczuk.** 1990. Direct enumeration of coliforms and *Escherichia coli* by hydrophobic grid membrane filter in 24 hours using MUG. J. Food Prot. **53**:948-952.
3. **Hartman, P.** 1988. MUG (glucuronidase) test for *Escherichia coli* in food and water. Proc. 5th International Symposium on Rapid Methods and Automation in Microbiology. Florence, Italy.
4. **Hitchins, A., P. Hartman, and E. Todd.** 1992. Coliforms-*Escherichia coli* and its toxins, p. 325-369. In C. Vanderzant and D. Splittstoesser (eds.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

EF-18 AGAR (6901)

Intended Use

EF-18 Agar is used for the selective and differential isolation of *Salmonella*, using the ISO-GRID Membrane Filtration System.

Product Summary and Explanation

EF-18 Agar was developed for the primary selective isolation of presumptive *Salmonella* spp. from enrichment broth using the ISO-GRID System. This medium suppresses growth of Gram-positive bacteria and certain Gram-negative bacteria, while enhancing growth of *Salmonella* spp. EF-18 Agar aids in differentiating *Salmonella* spp. from other *Enterobacteriaceae* based on lysine decarboxylase and sucrose fermentation reactions. EF-18 Agar is incubated at 42°C, providing additional selectivity against certain Gram-negative bacteria.

EF-18 Agar is recommended for the primary differential isolation and detection of *Salmonella* spp. from all foods using the ISO-GRID System.^{1,2,3}

Principles of the Procedure

Enzymatic Digest of Animal Tissue and Yeast Extract are the nitrogen and vitamin sources in EF-18 Agar. Dextrose and Sucrose are the fermentable carbohydrates. L-Lysine is a substrate for the lysine decarboxylase enzyme. Bile Salts, Sulfapyridine, and Novobiocin are selective agents. Bile Salts inhibit Gram-positive bacteria, Sulfapyridine and Novobiocin inhibit certain Gram-negative bacteria. Magnesium Sulfate aids in cell wall repair of injured *Salmonella* spp., enabling growth in the presence of Bile Salts. Bromthymol Blue is the pH indicator. Agar is the solidifying agent.

Bacteria that are able to grow on EF-18 Agar will ferment dextrose first, at a concentration of only 0.25% (w/v). Once the dextrose is depleted sucrose positive bacteria will ferment sucrose, producing a local pH drop in the colony and a color change to yellow in the pH indicator. Sucrose negative bacteria that are capable of producing the lysine decarboxylase enzyme will digest L-Lysine, increasing the pH, resulting in a color change to green, blue-green or blue in the pH indicator. Bacteria that are sucrose negative and lysine decarboxylase negative produce a mild local pH drop due to the initial fermentation of dextrose, causing a color change to yellow in the pH indicator. *Salmonella* spp. is sucrose negative and lysine decarboxylase positive resulting in green, blue-green, or blue colonies on EF-18 Agar.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	5 g
Yeast Extract.....	3 g
L-Lysine.....	10 g
Dextrose.....	2.5 g
Sucrose.....	15 g
Magnesium Sulfate	1.5 g
Bile Salts	1.5 g
Sulfapyridine.....	0.3 g
Bromthymol Blue.....	0.03 g
Novobiocin	0.015 g
Agar.....	15 g

Final pH: 6.8 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. Harmful. Maybe harmful if swallowed, inhaled, or absorbed through the skin.

Directions

1. Suspend 54 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and off-white to light beige.

Prepared Appearance: Prepared medium is clear to trace hazy, none to trace precipitate, and green.

Expected Cultural Response: Cultural response on EF-18 Agar, using the ISO-GRID Membrane Filtration System method. Inoculated EF-18 Agar was incubated at $42 \pm 0.5^{\circ}\text{C}$ for 18 - 24 hours.

Microorganism	Response	Reaction
<i>Klebsiella pneumoniae</i> QA-073	partial to complete inhibition	yellow colonies (if recovered)
<i>Salmonella hvittingfoss</i> QA-023	good growth	green colonies
<i>Salmonella infantis</i> QA-025	good growth	green colonies
<i>Salmonella typhimurium</i> QA-026	good growth	green colonies

Test Procedure

1. Prepare a sample homogenate and incubate in pre-enrichment broth.
2. Transfer 0.1 mL of the pre-enrichment culture into 10 mL of Tetrathionate Broth Base and incubate.
3. Filter 0.1 mL of Tetrathionate Broth Base culture through an ISO-GRID Hydrophobic Grid Membrane Filter.
4. Place the membrane filter on the surface of a predried EF-18 Agar.
5. Incubate inverted plate for 18 - 24 hours at 42°C .
6. Examine membrane filter for colonies.

Results

Presumptive *Salmonella* spp. colonies appear green, blue-green, or blue. If suspect colonies are present, subculture three representative colonies directly to biochemical screening media and purity plates for identification. If cultures are pure and reactions typical for *Salmonella*, proceed with serological confirmation.

Storage

Store sealed bottle containing the dehydrated medium at $2 - 30^{\circ}\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation some strains may grow poorly or fail to grow on this medium.

Packaging

EF-18 Agar **Code No.** **6901A** **500 g**

References

1. Entis, P. 1990. Improved hydrophobic grid membrane filter method, using EF-18 agar, for detection of *Salmonella* in foods: Collaborative study. J. AOAC. **73**:734-742.
2. Entis, P. 1996. Validation of the ISO-GRID 2-day rapid screening method for detection of *Salmonella* spp. in egg products. J. Food Prot. **59**:555-558.
3. Entis, P., and P. Boleszczuk. 1991. Rapid detection of *Salmonella* in foods using EF-18 agar in conjunction with the hydrophobic grid membrane filter. J. Food Prot. **54**:930-934.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (800) 783-3212 in the US/Canada or (410) 780-5120 or fax us at (800) 875-8563 in the US/Canada or (410) 780-5470.

LM-137 AGAR (6906)

Intended Use

LM-137 Agar is used with LM-137 Supplement for the presumptive enumeration of *Listeria* spp. using the ISO-GRID Membrane Filter System.

Product Summary and Explanation

LM-137 Agar was developed for the direct presumptive enumeration of *Listeria* spp., including *Listeria monocytogenes*, from food and environmental samples. This formula was designed to encourage rapid colony development of *Listeria* spp., while retarding or inhibiting growth of Gram-negative and Gram-positive competitors. LM-137 Agar is recommended for the direct presumptive enumeration of *Listeria* spp. and *L. monocytogenes*, from meats, poultry, dairy products, frozen eggs, and environmental samples using the ISO-GRID method.¹

Principles of the Procedure

Enzymatic Digest of Casein, Yeast Extract, Liver Peptone, and Egg Yolk emulsion are the nitrogen and vitamin sources in LM-137 Agar. Dextrose is the fermentable carbohydrate. Sodium Pyruvate is a supplementary energy source, and protects injured *Listeria* spp. from selective agents. Lithium Chloride, Acriflavin, and Di-Sodium Moxalactam (present in the LM-137 Supplement) inhibit or retard growth of many competing Gram-positive bacteria. Nalidixic Acid and Polymyxin B Sulfate (present in the LM-137 Supplement) inhibit or retard most Gram-negative bacteria. Magnesium Sulfate assists in cell wall repair and protects *Listeria* spp. from selective agents. Triphenyltetrazolium Chloride (present in the LM-137 Supplement) is a substrate for the tetrazolium reductase enzyme. Action of this enzyme results in the development of an insoluble red precipitate, producing a light pink color in *Listeria* spp. colonies. This pink color aids in the visualization and differentiation of the uninhibited competitors. Sodium Carbonate is the buffering agent. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Yeast Extract.....	1 g
Sodium Pyruvate.....	10 g
Lithium Chloride	5 g
Magnesium Sulfate	7.4 g
Dextrose.....	1 g
Sodium Carbonate	1 g
Acriflavin	0.015 g
Liver Peptone.....	10 g
Agar	15 g

Presterilized Supplements

Egg Yolk Emulsion (50%)
LM-137 Supplement

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. Harmful. Harmful if swallowed or inhaled. May cause eye burns.

Directions

1. Suspend 60.4 g of the medium in 950 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and aseptically add 50 mL of 50% Egg Yolk emulsion not containing tellurite and 10 mL of rehydrated LM-137 Supplement.
5. Mix thoroughly.
6. Determine pH and aseptically adjust, if necessary, to pH 7.4-7.5.
7. Pour into sterile petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light to medium tan.

Prepared Appearance: Prepared medium is clear to trace hazy and medium brown without supplements, yellow-brown with supplements.

Expected Cultural Response: Cultural response on LM-137 Agar, supplemented with Egg Yolk emulsion (50%) and LM-137 Supplement, using the ISO-GRID Filtration System method. Inoculated LM -137 Agar was incubated at 35°C for 18 - 48 hours.

Microorganism	Response	Reaction
<i>Listeria monocytogenes</i> ATCC® 153134	good growth	pink to dark pink-orange colonies
<i>Listeria monocytogenes</i> ATCC® 19111	good growth	pink to dark pink-orange colonies
<i>Listeria monocytogenes</i> ATCC® 7644	good growth	pink to dark pink-orange colonies
<i>Listeria monocytogenes</i> ATCC® 19112	good growth	pink to dark pink-orange colonies
<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Staphylococcus aureus</i> ATCC® 25923	suppressed	pale to dark orange

Test Procedure

1. Prepare a sample homogenate.
2. Filter 1 mL of the homogenate through the prefilter and ISO-GRID membrane filter.
3. Place the membrane filter on LM-137 Agar.
4. Incubate inverted plate for 22 - 24 hours at 35 - 37°C.
5. Examine membrane filter for colonies.

Results

Listeria spp. and/ or *Listeria monocytogenes* are homogeneous, pink to dark pink-orange, flat, small to medium-sized colonies. Any colonies of this type are a presumptive positive for *Listeria* spp. and/or *Listeria monocytogenes*. These presumptive colonies must be confirmed using procedures detailed in the ISO-GRID Methods Manual.

If positive colonies are present, count the number of positive squares (those containing one or more colonies). The number of positive squares is converted to the corresponding most probably number (MPN) using one of the methods described in the ISO-GRID Methods Manual.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

LM-137 Agar + Supplement Code No. 6906A 500 g

References

1. Entis, P. and I. Lerner. 2000. Twenty-four hour direct presumptive enumeration of *Listeria monocytogenes* in food and environmental samples using the ISO-GRID method with LM-137 Agar. J. Food Prot. **63**:354-363.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LMG AGAR (6902)

Intended Use

LMG Agar (Lactose Monensin Glucuronate Agar) is used in the selective and differential isolation of coliform bacteria, using the ISO-GRID Membrane Filtration System.

Product Summary and Explanation

Lactose Monensin Glucuronate Agar is based on the coliform enumeration medium, m-FC Agar.¹ The original m-FC formula was designed for fecal coliform enumeration from water samples using membrane filtration. This medium was modified for enumeration of total coliforms by eliminating rosolic acid supplement and reducing the incubation temperature. A formula change to modified m-FC Agar included the addition of Monensin to improve recovery of injured coliforms.²

LMG Agar is recommended for the detection and enumeration of total confirmed coliform bacteria from all foods using the ISO-GRID method.^{2,3}

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Yeast Extract are the nitrogen and vitamin sources in LMG Agar. Lactose is the fermentable carbohydrate. Monensin and Sodium Deoxycholate are selective agents, inhibiting Gram-positive bacteria and allowing Gram-negative organisms to grow. During Lactose fermentation a local pH drop in and around the colony causes a color change in the pH indicator, Aniline Blue. This color change results in the colony developing a blue, blue-green, or blue-white color. Lactose negative colonies metabolize nitrogen in the culture medium, resulting in a local increase in pH and a color change in the pH indicator to produce a pale yellow or beige colony. Glucuronic Acid induces *E. coli* to produce β -glucuronidase, and assists injured coliforms to develop in the presence of the selective agents. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	5 g
Yeast Extract.....	3 g
Lactose	12.5 g
Monensin	0.038 g
Sodium Deoxycholate	0.15 g
Aniline Blue	0.1 g
Glucuronic Acid, Sodium Salt	0.5 g
Agar	15 g

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. Irritant. Irritating to eyes, respiratory system, and skin at high concentrations.

Directions

1. Suspend 46.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.
4. Cool to 45 - 50°C.
5. Determine pH and adjust if necessary to 7.2 ± 0.2

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a greenish hint.

Prepared Appearance: Prepared medium is clear to trace hazy and medium blue to blue green.

Expected Cultural Response: Cultural response on LMG Agar, using the ISO-GRID Membrane Filtration System method. Inoculated LMG Agar was incubated at 35°C for 24 ± 2 hours.

Microorganism	Response	Reaction
<i>Bacillus subtilis</i> ATCC® 6633	inhibited	---
<i>Citrobacter freundii</i> ATCC® 8090	good growth	blue colonies
<i>Enterobacter agglomerans</i> QA-113	good growth	blue colonies
<i>Escherichia coli</i> ATCC® 25922	good growth	blue colonies
<i>Proteus vulgaris</i> ATCC® 13315	good growth	tan colonies

Test Procedure

1. Prepare a sample homogenate.
2. Filter 1 mL of the homogenate through the prefilter and ISO-GRID membrane filter.
3. Place the membrane filter on the surface of a predried LMG Agar plate.
4. Incubate inverted plate for 24 hours at 35 - 37°C.
5. Examine membrane filter for colonies.

Results

Coliforms appear as blue colonies. Any shade of blue is to be considered positive. If no blue colonies are present, the test for coliforms is complete; report should state "less than 10 coliforms/g".

If positive colonies are present, count the number of squares containing coliforms. Convert the number of squares to the corresponding MPN and calculate the Total Coliform Count, MPN using the methods described in the ISO-GRID Methods Manual.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation some strains of coliform bacteria may grow poorly or fail to demonstrate expected reactions on this medium.

Packaging

LMG Agar	Code No.	6902A	500 g

References

1. Brodsky, M. H., P. Entis, A. N. Sharpe, and G. A. Jarvis. 1982. Enumeration of indicator organisms in foods using the automated hydrophobic grid-membrane filter technique. J. Food Prod. **45**:292-296.
2. Entis, P., and P. Boleszczuk. 1990. Direct enumeration of coliforms and *Escherichia coli* by hydrophobic grid membrane filter in 24 hours using MUG. J. Food Prot. **53**:948-952.
3. Entis, P. 1989. Hydrophobic grid membrane filter/MUG method for total coliform and *Escherichia coli* enumeration in food: collaborative study. J. AOAC. **72**:936-950.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SCCRAM BROTH (6910)

Intended Use

SCCRAM Broth is a non-selective culture medium used for the rapid enrichment of *Salmonella* spp. with the ISO-GRID Membrane Filtration System.

Product Summary and Explanation

SCCRAM Broth was developed for the primary non-selective enrichment of *Salmonella* spp. from food samples. This medium was designed to stimulate sufficient repair and multiplication of *Salmonella* spp. within 6 - 8 hours of incubation at 42°C. The SCCRAM Broth formulation is used to enable direct presumptive detection of *Salmonella* in a total incubation period of 24 hours using EF-18 Agar and the ISO-GRID Membrane Filtration System.

Principles of the Procedure

Special Peptone is the nitrogen and vitamin source in SCCRAM Broth. Sodium Pyruvate is the carbohydrate source, and enhances recovery of injured *Salmonella* spp. Sodium Chloride maintains the osmotic balance of the medium. MOPS, free acid, and MOPS, sodium salt, are buffering agents.

Formula / Liter

Special Peptone	10 g
Sodium Pyruvate.....	5 g
Sodium Chloride	5 g
MOPS, free acid.....	10.5 g
MOPS, sodium salt	11.6 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. Irritant. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42.1 g of the medium in one liter of purified water.
2. If desired, add 10 g of Tween 20.
3. Stir without heating until dissolved completely.
4. Verify pH and adjust, if necessary.
5. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige to beige.

Prepared Appearance: Prepared medium is very light to light amber, trace to slightly hazy, none to very slight fine precipitate.

Expected Cultural Response: Cultural response in SCCRAM Broth incubated at 42 ± 0.5°C for 6 hours. After incubation in SCCRAM Broth, quality control organisms were processed using the ISO-GRID Filtration System. Filters were placed on EF-18 Agar and incubated at 42 ± 0.5°C for 18 - 24 hours.

Microorganism	Response	Reaction
<i>Salmonella cholerae-suis</i> QA-142	good growth	green colonies
<i>Salmonella newport</i> QA-197	good growth	green colonies
<i>Salmonella typhimurium</i> QA-045	good growth	green colonies

Test Procedure

1. Weigh a 25 g sample of food and homogenize in 225 mL of warm (42°C) SCCRAM Broth.
2. Incubate for 6 - 7 hours at 42°C.
3. Filter 1 mL of enrichment through an ISO-GRID Membrane Filter and place on EF-18 Agar.
4. Incubate for 18 - 24 hours at 42°C.
5. Examine the filter for presumptive positive viable *Salmonella* spp. colonies.

Results

Presumptive positive *Salmonella* spp. appear green, blue-green, or blue on EF-18 Agar. Further identification is required through biochemical and serological procedures.

If positive colonies are present, count the number of squares containing presumptive *Salmonella* spp. Convert the number of squares to the corresponding MPN and calculate the presumptive *Salmonella* spp. MPN, using the methods described in the ISO-GRID Methods Manual.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation some strains may grow poorly or fail to grow on this medium.

Packaging

SCCRAM Broth	Code No.	6910A	500 g

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SD-39 AGAR (6907)

Intended Use

SD-39 Agar is used in the presumptive enumeration of *Escherichia coli* O157:H7, and used with ISO-GRID Membrane Filtration System. SD-39 Agar conforms to AOAC.

Product Summary and Explanation

SD-39 Agar was formulated for direct presumptive enumeration of *Escherichia coli* O157:H7, and its direct differentiation from most other *E. coli*. SD-39 Agar combines some features of Lactose Monensin Glucuronate Agar and EF-18 Agar. Formula modifications include the addition of a chromogenic substrate for detection of β -glucuronidase activity, and substitution of sorbitol for lactose or sucrose as a fermentable carbohydrate. The formula also enables direct differential enumeration of β -glucuronidase-positive *E. coli*.

SD-39 Agar is recommended for the direct, presumptive enumeration of *E. coli* O157:H7 in meats, poultry, dairy foods, infant formula, liquid eggs, mayonnaise, and apple cider^{1,2}. This medium is also used for the direct enumeration of β -glucuronidase-positive *E. coli* from foods.³

Principles of the Procedure

Enzymatic Digest of Animal Tissue and Yeast Extract are the nitrogen and vitamin sources in SD-39 Agar. Dextrose and Sorbitol are the fermentable carbohydrates. Monensin and Sodium Deoxycholate are selective agent, inhibiting Gram-positive bacteria. Novobiocin inhibits or retards the growth of some Gram-negative bacteria, including *Klebsiella* spp. Magnesium Sulfate and Sodium Glucuronate assist in repair of injured bacteria, enabling injured *E. coli* to grow in the presence of selective agents. Sodium Chloride maintains the osmotic environment and assists *E. coli* O157:H7 to grow at the specified incubation of 44.0 - 44.5°C. Phenol Red is the pH indicator dye. X-Gluc is a chromogenic substrate for β -glucuronidase. L-Lysine is present to determine whether a strain is Lysine Decarboxylase positive or negative. Agar is the solidifying agent.

Bacteria able to grow on SD-39 Agar will ferment available dextrose first. Once dextrose has been depleted, sorbitol positive bacteria will begin to ferment sorbitol, producing a local pH drop in the colony and a color change to yellow in the pH indicator, Phenol Red. Sorbitol negative bacteria that are capable of producing the lysine decarboxylase enzyme will digest L-Lysine, producing a local pH rise in the colony and a color change to pink. Bacteria that are sorbitol negative and lysine decarboxylase negative produce a mild pH drop in the colony and a color change to yellow in the pH indicator. Glucuronidase positive *E. coli* will break down X-Gluc, resulting in the production of an insoluble blue precipitate in the colony. This will combine with the color of the pH indicator dye to produce a green colony in the case of sorbitol positive or lysine negative bacteria, or a purple colony in the case of an organism that is sorbitol negative and lysine positive. A typical colony of *E. coli* O157:H7 will produce a light pink to pink colony when isolated onto this medium in the ISO-Grid system.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	5 g
Yeast Extract.....	3 g
Sodium Chloride	5 g
L-Lysine.....	10 g
Dextrose.....	2.5 g
Sorbitol.....	20 g
Magnesium Sulfate	1.5 g
Monensin	0.038 g
Sodium Deoxycholate	0.15 g
Sodium Glucuronate	0.5 g
Novobiocin	0.0075 g
Phenol Red	0.12 g
X-Gluc.....	0.05 g
Agar	15 g

Final pH: 7.2 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. Harmful. May be harmful if swallowed, inhaled, or absorbed through the skin.

Directions

1. Suspend 63 g of the medium in one liter of purified water.
2. Heat with frequent agitation to boiling to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light to medium red-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and yellow-orange to light orange.

Expected Cultural Response: Cultural response on SD-39 Agar, using the ISO-GRID Membrane Filtration System method. Inoculated SD-39 Agar is incubated at 44.0 - 44.5°C for 22 - 24 hours.

Microorganism	Response	Reaction
<i>E. coli</i> O157:H7 QA-326	good growth	pink colonies
<i>E. coli</i> O157:H7 QA-330	good growth	light pink colonies
<i>E. coli</i> QA-059C	good growth	green colonies
<i>Klebsiella pneumoniae</i> QA-073	partial inhibition	light yellow colonies
<i>E. hermanii</i> QA-410	partial inhibition	light yellow colonies

Test Procedure

1. Prepare a sample homogenate in a specified diluent.
2. Filter 1 mL of the homogenate through the prefilter and ISO-GRID membrane filter.
3. Place the membrane filter on the surface of a predried SD-39 Agar plate.
4. Incubate inverted plate for 24 hours at 44.5°C.
5. Examine membrane filter for colonies.

Results

E. coli O157:H7 is β -glucuronidase negative, sorbitol negative, and lysine positive and produces pink colonies. Most other *E. coli* produce green colonies, and are β -glucuronidase positive and sorbitol positive. Occasional *E. coli* produce purple colonies, and are β -glucuronidase positive, sorbitol negative, and lysine negative.

If positive colonies are present, count the number of squares containing presumptive *E. coli* O157:H7. Convert the number of squares to the corresponding MPN and calculate the presumptive *E. coli* O157:H7 MPN, using the methods described in the ISO-GRID Methods Manual.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation some strains may grow poorly or fail to grow on this medium.

Packaging

SD-39 Agar **Code No.** **6907A** **500 g**

References

1. **Entis, P., and I. Lerner.** 1997. 24-Hour presumptive enumeration of *Escherichia coli* O157:H7 in food using the ISO-GRID method with SD-39 agar. J. Food Prot. **60**:883-890.
2. **Entis, P.** 1998. Direct 24-Hour presumptive enumeration of *Escherichia coli* O157:H7 in food using hydrophobic grid membrane filter, followed by serological confirmation: collaborative study. J. AOAC Int. **81**:403-418.
3. **Entis, P., and I. Lerner.** 1998. Enumeration of β -glucuronidase-positive *Escherichia coli* in foods by using the ISO-GRID method with SD-39 agar. J. Food Prot. **61**:913-916.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TSAF AGAR (6905)

Intended Use

TSAF Agar (Tryptone Soy Fast Green Agar) is used in determining total bacterial counts with the ISO-GRID Membrane Filtration System.

Product Summary and Explanation

Tryptone Soy Fast Green Agar is based on Tryptone Soy Agar. The original formula was modified by adding Fast Green FCF dye to enhance visibility of the colonies. TSAF Agar is recommended for the enumeration of total bacteria from all foods using the ISO-GRID Filtration Method.^{1,2}

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal are the nitrogen and vitamin sources in TSAF Agar. Sodium Chloride maintains the osmotic environment. Fast Green FCF stains all colonies green or blue-green, enhancing visibility. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein 15 g
Enzymatic Digest of Soybean Meal 5 g
Sodium Chloride 5 g
Fast Green (FCF) 0.25 g
Agar 15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. Harmful. Maybe harmful if swallowed or inhaled.

Directions

1. Suspend 40.3 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and green to blue-green-beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and blue-green to green.

Expected Cultural Response: Cultural response on TSAF incubated at 35°C for 48 ± 2 hours using the ISO-GRID Membrane Filtration System.

Microorganism	Response	Reaction
<i>Escherichia coli</i> ATCC® 25922	good growth	green colonies
<i>Staphylococcus aureus</i> ATCC® 25923	good growth	green colonies

Test Procedure

1. Prepare a sample homogenate.
2. Filter 1 mL of the homogenate through the prefilter and ISO-GRID membrane filter.
3. Place the membrane filter on the surface of a predried TSAF Agar plate.
4. Incubate inverted for 48 hours at 35 - 37°C.
5. Examine the membrane filter for green or blue colonies.

Results

Bacterial colonies stain green or blue.

If positive colonies are present, count the number of squares containing colonies. Convert the number of squares to the corresponding MPN and calculate the confirmed MPN, using the methods described in the ISO-GRID Methods Manual.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation some strains may grow poorly or fail to grow on this medium.

Packaging

TSAF AGAR	Code No.	6905A	500 g

References

1. **Entis, P.** 1986. Hydrophobic grid membrane filter method for aerobic plate count in foods: Collaborative study. J. AOAC **69**:671-676.
2. **Entis, P., and P. Boleszczuk.** 1986. Use of fast green FCF with tryptic soy agar for aerobic plate count by the hydrophobic grid membrane filter. J. Food Prot. **49**:278-279.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

YM-11 AGAR (6904)

Intended Use

YM-11 Agar is used for the selective enumeration of yeasts and molds using the ISO-GRID Membrane Filter System.

Product Summary and Explanation

YM-11 Agar was formulated to stimulate rapid growth and colonial development of a wide range of fungi. Peptone sources were selected to maximize growth in this medium. YM-11 Agar is buffered at a neutral pH, and selective agents were included to inhibit bacteria growth.

YM-11 Agar is recommended for the rapid enumeration of yeasts and molds in all foods using the ISO-GRID method.^{1,2}

Principles of the Procedure

Enzymatic Digest of Soybean Meal and Enzymatic Digest of Casein are the nitrogen and vitamin sources in YM-11 Agar. Dextrose is the fermentable carbohydrate. Sodium Chloride maintains the osmotic balance. Potassium Phosphate, Dibasic is a buffering agent. Trypan Blue is a nontoxic dye, staining yeasts and molds colonies blue to enhance their visibility.³ Chloramphenicol and Chlortetracycline-HCl are selective agents used to inhibit bacterial growth. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Soybean Meal	20 g
Enzymatic Digest of Casein	20 g
Dextrose.....	5 g
Sodium Chloride	5 g
Potassium Phosphate, Dibasic	2.4 g
Trypan Blue.....	0.03 g
Chloramphenicol.....	0.1 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Presterilized Antibiotic Supplement

Chlortetracycline-HCl, 20 mL

Precautions

1. For Laboratory Use.
2. Very toxic. Toxic by inhalation and contact with skin.

Directions

1. Suspend 67.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C. Aseptically add 20 mL of a 0.5% (wt/v) aqueous solution of Chlortetracycline HCl.
5. Mix thoroughly.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and tan.

Prepared Appearance: Prepared medium is clear to trace hazy and dark blue-grey.

Expected Cultural Response: Cultural response on YM-11 Agar, supplemented with Chlortetracycline-HCl, using the ISO-GRID Filtration System method. Inoculated YM-11 Agar was incubated at 25°C for up to 52 hours.

Microorganism	Response	Reaction
<i>Aspergillus niger</i> ATCC® 16404	good growth	blue colonies
<i>Saccharomyces cerevisiae</i> ATCC® 9763	good growth	blue colonies
<i>Bacillus subtilis</i> QA-057	inhibited	---
<i>Escherichia coli</i> ATCC® 25922	inhibited	---

Test Procedure

1. Prepare a sample homogenate.
2. Filter 1 mL of the homogenate through the prefilter and ISO-GRID membrane filter.
3. Place the membrane filter on the surface of a predried YM-11 Agar plate.
4. Incubate inverted plate for 48 - 52 hours at 25°C.
5. Examine membrane filter for colonies.

Results

Yeast colonies appear blue. Molds appear blue-grey. If no blue or blue-grey colonies are present, the test for yeast and molds is complete. Results are reported as less than 10 yeasts and mold per gram.

If positive colonies are present, count the number of positive squares (those containing one or more colonies). The number of positive squares are converted to the corresponding most probably number (MPN) using one of the methods described in the ISO-GRID Methods Manual, and the yeast and mold MPN per gram is calculated.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi. Non-selective fungal media should be used concurrently with selective media when isolating fungi.

Packaging

YM-11 Agar **Code No.** **6904A** **500 g**

References

1. **Entis, P.** 1996. Two-day hydrophobic grid membrane filter method for yeast and mold enumeration in food using YM-11 Agar: collaborative study. J. AOAC Int. **79**:1069-1082.
2. **Entis, P., and I. Lerner.** 1996. Two-day yeast and mold enumeration using the ISO-GRID membrane filter system in conjunction with YM-11 Agar. J. Food Prot. **59**:416-419.
3. **Lin, C.C.S., D. Y. C. Fung, and P. Entis.** 1984. Growth of yeast and mold on Trypan Blue Agar in conjunction with the ISO-GRID system. Can J. Microbiol. **30**:1405-1407.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

KLIGLER IRON AGAR (7140)

Intended Use

Kligler Iron Agar is used for the differentiation of microorganisms on the basis of dextrose and lactose fermentation and hydrogen sulfide production.

Product Summary and Explanation

Kligler Iron Agar is a modification of Kligler's original formula, recommended to identify pure cultures of colonies picked from primary plating media.¹ The original medium was a soft nutrient agar containing dextrose, Andrade indicator and lead acetate.¹ Russell devised a medium containing glucose, lactose, and an indicator for the differentiation of lactose-fermenting and nonlactose-fermenting gram-negative bacilli.² Kligler found lead acetate could detect hydrogen sulfide when combined with Russell double sugar medium for the differentiation of typhoid, paratyphoid, and dysentery groups.³ Bailey and Lacy simplified the formula by using phenol red as the pH indicator instead of Andrade indicator. A similar medium containing sucrose, tryptone, ferrous sulfate, and thiosulfate was developed by Sulkins and Willet.⁵

Kligler Iron Agar is recommended for differentiation of enteric gram-negative bacilli from clinical specimens^{6,7} and food samples.⁸

Principles of the Procedure

Kligler Iron Agar combines the principles of Russell double sugar medium and lead acetate agar into one medium. This combination permits differentiation of gram-negative bacilli by their ability to ferment Dextrose or Lactose, and produce hydrogen sulfide. Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and vitamins required for organism growth. Ferric Ammonium Citrate and Sodium Thiosulfate are indicators of hydrogen sulfide production. Phenol Red is the pH indicator. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein.....	10 g
Enzymatic Digest of Animal Tissue	10 g
Lactose	10 g
Dextrose	1 g
Ferric Ammonium Citrate	0.5 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.5 g
Phenol Red	0.025 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Distribute into test tubes and autoclave for 15 minutes at 121°C.
4. After autoclaving, allow medium to solidify in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is reddish-orange to red, trace to slightly hazy.

Expected Cultural Response: Cultural response in Kligler Iron Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions			
		Slant	Butt	Gas	H ₂ S
<i>Escherichia coli</i> ATCC® 25922	growth	A	A	+	--
<i>Proteus mirabilis</i> ATCC® 12453	growth	K	A	--	+
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	K	K	--	--
<i>Salmonella typhimurium</i> ATCC® 14028	growth	K	A	+/-	+
<i>Shigella flexneri</i> ATCC® 12022	growth	K	A	--	--

The organisms listed are the minimum that should be used for quality control testing.

KEY: A, acid, K, alkaline, +, positive, -, negative, +/-, usually positive

Test Procedure

Stab the center of the medium into tube butt. Withdraw the needle, and streak surface of the slant. Loosen caps to allow a free exchange of air before incubating at 35°C for 18 – 38 hours. Read tubes for acid production on slant/butt, gas production, and hydrogen sulfide production.

Results

An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose and lactose. An alkaline slant-alkaline butt (red/red) indicates dextrose and lactose did not ferment (non-fermenter). Cracks, splits, or bubbles in the medium indicate gas production. A black precipitate in butt indicates hydrogen sulfide production.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Hydrogen sulfide producing organisms may produce a black precipitate to such a degree that the reaction in the butt is completely masked. If hydrogen sulfide is produced, dextrose is fermented even if it is not observed.⁹
2. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.
3. Pure cultures are essential when inoculating Kligler Iron Agar. If inoculated with a mixed culture, irregular observations may occur.
4. Hydrogen sulfide determinations using Kligler Iron Agar should be limited to members of *Enterobacteriaceae*.⁹

Packaging

Kligler Iron Agar	Code No.	7140A	500 g
		7140B	2 kg
		7140C	10 kg

References

1. **Kligler, I. J.** 1917. A simple medium for the differentiation of members of the typhoid-paratyphoid group. *Am. J. Public Health* **7**:1042-1044.
2. **Russell, F. F.** 1911. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. *J. Med. Res.* **25**:217.
3. **Kligler, I. J.** 1918. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. *J. Exp. Med.* **28**:319-322.
4. **Bailey, S. F., and L. R. Lacy.** 1927. A modification of the Kligler lead acetate medium. *J. Bacteriol.* **13**:183.
5. **Sulkin, S. E., and J. C. Willett.** 1940. A triple sugar-ferrous sulfate medium for use in identification of enteric organisms. *J. Lab. Clin. Med.* **25**:649-653.
6. **Isenberg, H. D. (ed.).** *Clinical microbiology procedures handbook*, vol.1. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D. C.
8. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, M.D.
9. **MacFaddin, J. F.** *Media for isolation-cultivation-identification-maintenance of medial bacteria*, Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LACTOBACILLI MRS AGAR (7543)

Intended Use

Lactobacilli MRS Agar is used for the cultivation of lactobacilli.

Product Summary and Explanation

Lactobacilli MRS Agar is based on the formulations of deMan, Rogosa and Sharpe (MRS).¹ This medium supports luxuriant growth of lactobacilli from oral, fecal, dairy, and other sources.

Principles of the Procedure

Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract are the carbon, nitrogen, and vitamin sources used to satisfy general growth requirements in Lactobacilli MRS Broth. Dextrose is the fermentable carbohydrate. Sodium Acetate is an inhibitory agent. Sodium Acetate and Ammonium Citrate act as selective agents as well as energy sources. Potassium Phosphate is the buffering agent. Magnesium Sulfate and Manganese Sulfate provide cations used in metabolism. Polysorbate 80 is a surfactant, facilitating uptake of nutrients by lactobacilli. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	10 g
Beef Extract	10 g
Yeast Extract.....	5 g
Dextrose.....	20 g
Sodium Acetate.....	5 g
Polysorbate 80	1 g
Potassium Phosphate	2 g
Ammonium Citrate	2 g
Magnesium Sulfate	0.1 g
Manganese Sulfate	0.05 g
Agar	15 g

Final pH: 6.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 70 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous with soft lumps and yellow-tan.

Prepared Appearance: Prepared medium is light beige.

Expected Cultural Response: Cultural response on Lactobacillus MRS Agar at 35°C after 24 - 96 hours incubation.

Microorganism	Response
<i>Lactobacillus casei</i> ATCC® 393	growth
<i>Lactobacillus fermentum</i> ATCC® 9338	growth
<i>Lactobacillus plantarum</i> ATCC® 8014	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

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 inoculum throughout medium by rotating the plate in one direction, then in the reverse direction.
3. Allow 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.

Results

Lactobacilli appear as large, white colonies embedded in or on Lactobacilli MRS Agar. Growth can be subcultured onto appropriate media for use in additional procedures. Refer to appropriate references for recommendation on the identification of *Lactobacillus* spp.²⁻⁴

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Organisms other than lactobacilli may grow in this medium. Isolates must be confirmed as lactobacilli by appropriate biochemical testing.

Packaging

Lactobacilli MRS Agar	Code No.	7543A	500 g
		7543B	2 kg
		7543C	10 kg

References

1. **deMan, J. C., M. Rogosa, and M. E. Sharpe.** 1960. A medium for the cultivation of lactobacilli. *J. Bacteriol.* **23**:130.
2. **MacFaddin, J. F.** 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol. 1 Williams & Wilkins, Baltimore, MD.
3. **Vanderzant, C. and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LACTOBACILLI MRS BROTH (7406)

Intended Use

Lactobacilli MRS Broth is used for the cultivation of lactobacilli.

Product Summary and Explanation

Lactobacilli MRS Broth is based on the formulations of deMan, Rogosa and Sharpe (MRS).¹ This medium supports luxuriant growth of lactobacilli from oral, fecal, dairy, and other sources.

Principles of the Procedure

Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract are the carbon, nitrogen, and vitamin sources used to satisfy general growth requirements in Lactobacilli MRS Broth. Dextrose is the fermentable carbohydrate. Sodium Acetate is an inhibitory agent. Sodium Acetate and Ammonium Citrate act as selective agents as well as energy sources. Potassium Phosphate is the buffering agent. Magnesium Sulfate and Manganese Sulfate provide cations used in metabolism. Polysorbate 80 is a surfactant, facilitating uptake of nutrients by lactobacilli.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	10 g
Beef Extract	10 g
Yeast Extract.....	5 g
Dextrose.....	20 g
Sodium Acetate.....	5 g
Polysorbate 80	1 g
Potassium Phosphate	2 g
Ammonium Citrate	2 g
Magnesium Sulfate	0.1 g
Manganese Sulfate	0.05 g

Final pH: 6.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Dissolve 55 g of the medium in one liter of purified water.
2. Heat with frequent agitation to boiling to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous with soft lumps and yellow-tan.

Prepared Appearance: Prepared medium is clear.

Expected Cultural Response: Cultural response in Lactobacillus MRS Broth at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Lactobacillus casei</i> ATCC® 393	growth
<i>Lactobacillus fermentum</i> ATCC® 9338	growth
<i>Lactobacillus plantarum</i> ATCC® 8014	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Samples can be inoculated directly into Lactobacillus 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.

Results

Growth of *Lactobacillus* spp. appear turbid. Growth can be subcultured onto appropriate media for use in additional procedures. Refer to appropriate references for recommendation on the identification of *Lactobacillus* spp.²⁻⁴

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Organisms other than lactobacilli may grow in this medium. Isolates must be confirmed as lactobacilli by appropriate biochemical testing.

Packaging

Lactobacilli MRS Broth	Code No.	7406A	500 g
		7406B	2 kg
		7406C	10 kg

References

1. **deMan, J. C., M. Rogosa, and M. E. Sharpe.** 1960. A medium for the cultivation of lactobacilli. J. Bacteriol. **23**:130.
2. **MacFaddin, J. F.** 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol. 1 Williams & Wilkins, Baltimore, MD.
3. **Vanderzant, C. and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

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LACTOBACILLUS SELECTIVE AGAR BASE (7234)

Intended Use

Lactobacillus Selective Agar Base is used for the isolation and enumeration of lactobacilli.

Product Summary and Explanation

Lactobacillus Selective Agar Base was developed by Rogosa, Mitchell, and Wiseman.^{1,2} This medium is used for isolation, enumeration, and identification of lactobacilli in oral specimens, feces, vaginal cultures, and foods.^{3,4} The low pH and high acetate concentrations effectively suppress other bacterial flora allowing lactobacilli to flourish.

Principles of the Procedure

Enzymatic Digest of Casein provides carbon, nitrogen, and amino acids used to support general growth requirements in Lactobacillus Selective Agar Base. Yeast Extract is a major source of vitamins. Dextrose is a carbohydrate. Sodium Acetate Hydrate and Ammonium Citrate inhibit streptococci, molds, and other oral microbial flora and restrict swarming. Monopotassium Phosphate is the buffering agent. Magnesium Sulfate, Manganese Sulfate and Ferrous Sulfate are sources of inorganic ions. Polysorbate 80 acts as a surfactant. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Yeast Extract.....	5 g
Monopotassium Phosphate	6 g
Ammonium Citrate	2 g
Dextrose.....	20 g
Sodium Acetate Hydrate	25 g
Magnesium Sulfate	0.575 g
Manganese Sulfate	0.12 g
Ferrous Sulfate	0.034 g
Polysorbate 80	1 g
Agar	15 g

Final pH: 5.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Glacial Acetic Acid, 1.32 mL

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. May cause irritation to skin, eyes, and respiratory tract.

Directions

1. Suspend 84 g of the medium in one liter of purified water. Mix thoroughly.
2. Add 1.32 mL of glacial acetic acid.
3. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
4. Avoid overheating. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige to amber.

Prepared Appearance: Prepared medium is hazy and light beige to amber.

Expected Cultural Response: Cultural response on Lactobacillus Selective Agar Base at 35°C after 48 - 98 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Lactobacillus casei</i> ATCC® 393	growth
<i>Lactobacillus fermentum</i> ATCC® 9338	growth
<i>Lactobacillus plantarum</i> ATCC® 8014	growth
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Organisms other than lactobacilli may grow on this medium. Isolates must be confirmed by appropriate biochemical tests.

Packaging

Lactobacillus Selective Agar Base	Code No.	7234A	500 g
		7234B	2 kg
		7234C	10 kg

References

1. **Rogosa, M., J. A. Mitchell, and R. F. Wiseman.** 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. *J. Bacteriol.* **62**:132.
2. **Rogosa, M., J. A. Mitchell, and R. F. Wiseman.** 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. *J. Dental Res.* **30**:682.
3. **Vedamuthu, E. R., M. Raccach, B. A. Glatz, E. W. Seitz, and M. S. Reddy.** 1992. Acid-producing microorganisms. *In* C. Vanderzant, and D. F. Splittstoesser (eds.). *Compendium of methods for the microbiological examination of foods*, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LACTOSE BROTH (7141)

Intended Use

Lactose Broth is used for the cultivation of salmonella and coliform bacteria from food, dairy, water and pharmaceutical products.

Product Summary and Explanation

Lactose Broth is frequently used as a pre-enrichment medium when testing foods and dairy products for *Salmonella* spp. In dried or processed foods, *Salmonella* species may be sublethally injured and in low numbers. The presence of other bacteria as well as components of the food sample may hinder growth and recovery of *Salmonella*. Pre-enrichment in a nonselective medium such as Lactose Broth allows for repair of cell damage, dilutes toxic or inhibitory substances, and provides a nutritional advantage to *Salmonella* over other bacteria.¹ Lactose Broth is widely used and is included in many procedures for testing foods, dairy products and other materials.

Lactose Broth is also used for the detection of coliform organisms in water, dairy products and other materials.¹⁻⁵

Principles of the Procedure

Enzymatic Digest of Gelatin and Beef Extract provide the carbon and nitrogen sources for general growth requirements in Lactose Broth. Lactose is a carbohydrate source. Fermentation of lactose is demonstrated by the production of gas.

Formula / Liter

Enzymatic Digest of Gelatin 5 g
Beef Extract 3 g
Lactose 5 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 13 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light-beige.

Prepared Appearance: Prepared medium is pale to light yellow and clear.

Expected Cultural Response: Cultural response in Lactose Broth at 35°C after 18 - 48 hours incubation.

Microorganism	Reaction	Reaction (Gas)
<i>Enterococcus faecalis</i> ATCC® 19433	good growth	----
<i>Escherichia coli</i> ATCC® 25922	good growth	positive
<i>Klebsiella pneumoniae</i> ATCC® 13883	good growth	positive
<i>Pseudomonas aeruginosa</i> ATCC® 27853	good growth	----
<i>Salmonella typhimurium</i> ATCC® 14028	good growth	----

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Lactose Broth is used in the pre-enrichment phase of the preparation of food samples for isolation of *Salmonella* spp. Consult appropriate references for specific procedures for each type of material being tested.¹⁻⁴

1. Transfer a 25 g or 25 mL sample of test material into a container. Add 225 mL of sterile Lactose Broth. Mix as necessary to make a homogeneous suspension. Incubate at 35°C for 24 hours.
2. Transfer 1 mL of suspension to appropriate enrichment broths, such as Tetrathionate Broth and Selenite Cystine Broth. Incubate at 35°C for 24 hours.
3. Transfer a loopful of suspension to appropriate selective agar media, such as Hektoen Enteric Agar, XLD Agar and Bismuth Sulfite Agar. Incubate at 35°C for 24 hours.

Results

Pre-enrichment, selective enrichment and selective plating increase the likelihood of isolating *Salmonella* from foods and other materials.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Lactose Broth	Code No.	7141A	500 g
		7141B	2 kg
		7141C	10 kg

References

1. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
4. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
5. **Eaton, A.D., L.S. Clesceri, and A.E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LAURYL SULFATE BROTH (7142)

Intended Use

Lauryl Sulfate Broth is used for the detection of coliform bacteria in water and wastewater.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative anaerobic, gram-negative, non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours.¹ Members of the *Enterobacteriaceae* comprise the majority of this group, but organisms such as *Aeromonas* spp. may also be included. Procedures to detect and confirm coliforms are used in testing water, foods, dairy products, and other materials.¹⁻⁴

Lauryl Sulfate Broth, also referred to as Lauryl Tryptose Broth, is prepared according to the formula of Mallmann and Darby.⁵ During their investigation, Sodium Lauryl Sulfate produced the best results for inhibition of organisms other than coliforms.⁵ Lauryl Sulfate Broth, abbreviated as LSB, is used in the presumptive phase of the Standard Total Coliform Fermentation Technique in the examination of water,² and coliform detection of foods.^{3,4,6}

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, vitamins, minerals, and amino acids in Lauryl Tryptose Broth. Lactose is the fermentable carbohydrate for coliforms. Potassium Phosphates are the buffering agents, and Sodium Chloride is used to maintain the osmotic balance of the medium. Sodium Lauryl Sulfate is the selective agent used to inhibit non-coliform organisms.

Formula / Liter

Enzymatic Digest of Casein	20 g
Lactose	5 g
Sodium Chloride	5 g
Monopotassium Phosphate	2.75 g
Disodium Phosphate.....	2.75 g
Sodium Lauryl Sulfate	0.1 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 35.6 g of the medium in one liter of purified water.
2. Prepare double strength broth for evaluating 10 mL samples.
3. Distribute into tubes containing inverted fermentation Durham tubes. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and white.

Prepared Appearance: Prepared medium is yellow to gold and clear to trace hazy.

Expected Cultural Response: Cultural response in Lauryl Sulfate Broth at 35°C after 24 hours incubation.

Microorganism	Response	Reaction (Gas)
<i>Escherichia coli</i> ATCC® 25922	good growth	positive
<i>Proteus mirabilis</i> ATCC® 12453	good growth	negative
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	----

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Follow the methods and procedures for the detection of coliform organisms as described in standard methods.^{1-4,6}

Results

After incubation of the tubes at 35°C for 24 hours, examine for turbidity and gas production. If no gas has formed in the inverted tube, reincubate and reexamine after 48 hours.^{2,3}

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Lauryl Sulfate Broth	Code No.	7142A	500 g
		7142B	2 kg
		7142C	10 kg

References

1. **Marshall, R. T. (ed.).** 1992. Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **U. S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Mallmann, W. L., and C. W. Darby.** 1941. Uses of a lauryl sulphate tryptose broth for the detection of coliform organisms. Am J. Public Health. 31:127.
6. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LAURYL SULFATE BROTH w/ MUG (7300)

Intended Use

Lauryl Sulfate Broth w/ MUG is used for the detection of coliforms and the fluorogenic detection of *Escherichia coli*.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative anaerobic, Gram-negative, non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours.¹ Members of the *Enterobacteriaceae* comprise the majority of this group, but organisms such as *Aeromonas* spp. may also be included. Procedures to detect and confirm coliforms are used in testing water, foods, dairy products, and other materials.¹⁻⁴

Lauryl Sulfate Broth, also referred to as Lauryl Tryptose Broth, is prepared according to the formula of Mallmann and Darby.⁵ During their investigation, Sodium Lauryl Sulfate produced the best results for inhibition of organisms other than coliforms.⁵ Feng and Hartman⁶ 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 Sulfate Broth. Incorporating MUG into Lauryl Sulfate Broth (LSB) permits the detection of *E. coli* among the coliform colonies.^{3,4}

LSB w/ MUG is recommended by the American Public Health Association (APHA) and the Association of Official Analytical Chemists (AOAC).^{3,4,6}

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, vitamins, minerals, and amino acids in Lauryl Tryptose Broth. Lactose is the fermentable carbohydrate for coliforms. Potassium Phosphates are the buffering agents, and Sodium Chloride is used to maintain the osmotic balance of the medium. Sodium Lauryl Sulfate is the selective agent used to inhibit non-coliform organisms.

The addition of MUG (4-methylumbelliferyl-β-D-glucuronide) provides another criterion to determine the presence of *E. coli* in food and environmental samples. *E. coli* produces the enzyme glucuronidase that hydrolyzes MUG to yield a fluorogenic product that is detectable under long-wave (366 nm) UV light.

Formula / Liter

Enzymatic Digest of Casein	20 g
Lactose	5 g
Monopotassium Phosphate	2.75 g
Dipotassium Phosphate	2.75 g
Sodium Chloride	5 g
Sodium Lauryl Sulfate	0.1 g
4-Methylumbelliferyl-β-D-glucuronide	0.05 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 35.7 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into tubes containing inverted fermentation Durham tubes. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and white.

Prepared Appearance: Prepared medium is yellow to gold and clear to trace hazy.

Expected Cultural Response: Cultural response in Lauryl Sulfate Broth w/ MUG at 35°C after 24 hours incubation.

Microorganism	Response	Reactions	
		Gas	Fluorescence
<i>Enterobacter aerogenes</i> ATCC® 13048	good growth	positive	negative
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	----	----
<i>Escherichia coli</i> ATCC® 25922	good growth	positive	positive
<i>Proteus mirabilis</i> ATCC® 12453	good growth	negative	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Lauryl Sulfate Broth w/ MUG.^{3,4,6}

Results

After incubation of the tubes at 35°C for 24 hours, examine for turbidity, gas production, and fluorescence. 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.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may grow poorly or fail to grow on this medium.
2. Strains of *E. coli* that fail to grow in LSB w/ MUG, fail to produce gas, or fail to produce glucuronidase may infrequently be encountered. 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; gas production, growth at 44°C.

Packaging

Lauryl Sulfate Broth w/ MUG	Code No.	7300A	500 g
		7300B	2 kg
		7300C	10 kg

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **U.S. and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Mallmann, W. L., and C. W. Darby.** 1941. Uses of a lauryl sulphate tryptose broth for the detection of coliform organisms. Am J. Public Health. **31**:127.
6. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43**:1320-1329.
7. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LB AGAR (7289) (LENNOX L AGAR)

Intended Use

LB Agar (Lennox L Agar) is used in molecular genetic studies.

Product Summary and Explanation

LB Agar is nutritionally rich, developed by Lennox for 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 provides all nutritional requirements of these organisms. LB Agar contains half the sodium chloride level of the Miller formulation of LB Agar, allowing the researcher to select the optimal salt concentration for a specific strain.²

Principles of the Procedure

The nitrogen, amino acids, and carbon sources are provided by Enzymatic Digest of Casein. Vitamins and certain trace elements are supplied by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein 10 g
Yeast Extract..... 5 g
Sodium Chloride 5 g
Agar 12 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 32 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to moderately hazy and yellow to gold, may have a slight precipitate.

Expected Cultural Response: Cultural response on LB Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.²

Results

After sufficient incubation, the medium should show growth as evidenced by formation of colonies and/or a confluent lawn of growth.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

LB Agar (Lennox L Agar)	Code No.	7289A	500 g
		7289B	2 kg
		7289C	10 kg

References

1. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology*. **1**:190.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1994. Current protocols in molecular biology, vol. 1. Current Protocols, New York, N.Y.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LB BROTH (7290) (LENNOX L BROTH)

Intended Use

LB Broth (Lennox L Broth) is used in molecular genetic studies.

Product Summary and Explanation

LB Broth formula is based on L Broth described by Lennox for growth and maintenance of *E. coli* strains used in molecular microbiology procedures.¹ LB Broth is nutritionally rich, formulated for the isolation of pure recombinant strains. *E. coli* is grown to late log phase in LB Broth. Some plasmid vectors may replicate to high copy numbers without selective amplification. Some vectors may require selective amplification to reach high copy numbers. Chloramphenicol can be added to inhibit host synthesis and, as a result, prevent replication of the bacterial chromosome.²

LB Broth contains ten times the sodium chloride level of Luria Broth, Miller and one half of that found in LB Broth, Miller.³ This permits 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.

Principles of the Procedure

The nitrogen, amino acids, and carbon sources are provided by Enzymatic Digest of Casein. Vitamins and certain trace elements are supplied by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride.

Formula / Liter

Enzymatic Digest of Casein 10 g
Yeast Extract..... 5 g
Sodium Chloride 5 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 20 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is yellow to gold and clear to moderately hazy.

Expected Cultural Response: Cultural response on LB Broth at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.¹⁻³

Results

Growth is evident in the form of turbidity.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

LB Broth (Lennox L Broth)	Code No.	7290A	500 g
		7290B	2 kg
		7290C	10 kg

References

1. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology*. **1**:190.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
3. **Miller, J. H.** 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LETTEEN AGAR BASE (7118)

Intended Use

Letheen Agar Base is used with Polysorbate 80 for the testing of quaternary ammonium compounds for antimicrobial activity.

Product Summary and Explanation

In 1948, Weber and Black described the value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers.¹ The addition of Lecithin and Polysorbate 80 to Tryptone Glucose Extract (TGE) Agar resulted in a medium that effectively neutralizes quaternary ammonium compounds while testing germicidal activity. Letheen Agar Base is a modification of TGE Agar, with the addition of Lecithin and Polysorbate 80.

Letheen Agar Base is specified for use by the American Society for Testing Materials (ASTM) in Standard Test Method for Preservatives in Water-Containing Cosmetics.² Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test.

Principles of the Procedure

Enzymatic Digest of Casein and Beef Extract provide nitrogen, carbon, vitamins, and minerals in Letheen Agar Base. Dextrose is the fermentable carbohydrate. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with Lecithin, ethanol.^{3,4,5,6} Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Dextrose.....	1 g
Beef Extract	3 g
Lecithin.....	1 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / Liter

Polysorbate 80, 7 g

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 25 g of the medium and 7 g of Polysorbate 80 in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium yellow, and trace to slightly hazy.

Expected Cultural Response: Cultural response on Letheen Agar Base at 35°C after 24 - 48 hours incubation.

Microorganism	Response
<i>Enterococcus faecalis</i> ATCC® 29212	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Lethen Agar Base is used in a variety of procedures. Consult appropriate references for complete information.^{4,7}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Lethen Agar Base	Code No.	7118A	500 g
		7118B	2 kg
		7118C	10 kg

References

1. **Weber, G. R., and L. A. Black.** 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. **24**:134-139.
2. **American Society for Testing Materials.** 1991. Standard test method for preservatives in water-containing cosmetics, E 640-78. Annual Book of ASTM Standards, Philadelphia, PA.
3. **Quisno, R., I. W. Gibby, and M. J. Foter.** 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118**:320-323.
4. **Erlandson, A. L., Jr., and C. A. Lawrence.** 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science. **118**:274-276.
5. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. Appl. Environ. Microbiol. **32**:80-84.
6. **Favero (chm.).** 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.
7. **Association of Official Analytical Chemists.** 1995. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LETTEEN AGAR BASE, MODIFIED (7495)

Intended Use

Letheen Agar Base, Modified is used with Polysorbate 80 for the isolation of microorganisms from cosmetics.

Product Summary and Explanation

In 1948, Weber and Black described the value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers.¹ The addition of Lecithin and Polysorbate 80 to Tryptone Glucose Extract (TGE) Agar resulted in a medium that effectively neutralizes quaternary ammonium compounds in testing of germicidal activity. Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test.

Letheen Agar Base, Modified is based on the formula described in FDA Bacteriological Analytical Manual, and a modification of Letheen Agar Base.² Letheen Agar Base, Modified is recommended by the FDA for use in the microbiological testing of cosmetics.³

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen and carbon required for good growth of a wide variety of bacteria and fungi. The nitrogen level was increased in Letheen Agar Base, Modified to provide better growth. Yeast Extract provides vitamins and cofactors in this medium. Sodium Chloride maintains the osmotic balance. Sodium Bisulfite, Polysorbate 80, and Lecithin (present in Letheen Agar Base) neutralizes quaternary ammonium compounds. Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with Lecithin, ethanol.⁴⁻⁷ These preservatives are commonly used in the cosmetic industry. Agar is the solidifying agent.

Formula / Liter

Letheen Agar Base	25 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	10 g
Yeast Extract.....	2 g
Sodium Chloride	5 g
Sodium Bisulfite	0.1 g
Agar	15 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / Liter

Polysorbate 80, 7 g

Precautions

1. For Laboratory Use.
2. HARMFUL. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 52.1 g of the medium and 7 g of Polysorbate 80 in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium yellow and trace to moderately hazy.

Expected Cultural Response: Cultural response on Lethen Agar Base, Modified at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Enterococcus faecalis</i> ATCC® 29212	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure³

1. Prepare and dilute samples in Lethen Broth Base, Modified in accordance with established guidelines.
2. Using the spread plate technique, inoculate in duplicate 0.1 mL of the diluted samples onto Lethen Agar Base, Modified, Potato Dextrose Agar containing Chlortetracycline, Baird Parker Agar, Anaerobic Agar, and a second set of Lethen Agar Base, Modified plates.
3. Incubate one set of Lethen Agar Base, Modified at 30°C for 48 hours and the other set at 35°C under anaerobic conditions for 2 - 4 days. Incubate the Potato Dextrose Agar plates at 30°C for 7 days and the Baird Parker Agar plates, if inoculated, at 35°C for 48 hours.
4. Incubate diluted samples from Step 1 at 35°C for 7 days. Subculture enriched samples onto Lethen Agar Base, Modified only if there is no growth on the primary Lethen Agar Base, Modified.

Results

Examine plates for evidence of growth and characteristic colonial morphology. Determine colony counts and subculture each colony type onto Lethen Agar Base, Modified and MacConkey Agar (also Baird Parker if used in Step 2).

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Lethen Agar Base, Modified	Code No.	7495A	500 g
		7495B	2 kg
		7495C	10 kg

References

1. **Weber, G. R., and L. A. Black.** 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. **24**:134-139.
2. **Tomlinson, L. (ed.).** 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
3. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1992. In Tomlinson, L. A. (ed.). FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
4. **Quisno, R., I. W. Gibby, and M. J. Foter.** 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118**:320-323.
5. **Erlandson, A. L., Jr., and C. A. Lawrence.** 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science. **118**:274-276.
6. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. Appl. Environ. Microbiol. **32**:80-84.
7. **Favero (chm.).** 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Technical Information

Contact Acumedia Manufacturers, Inc. at TEL (800)783-3213 in the US/Canada or (410)780-5120 and FAX (800)875-8563 in the US/Canada or (410)780-5470 for Technical Service on questions involving dehydrated culture media preparation or performance.

LETHEEN BROTH BASE (7105)

Intended Use

Letheen Broth Base is used with Polysorbate 80 for the testing of quaternary ammonium compounds for antimicrobial activity.

Product Summary and Explanation

In 1948, Weber and Black described the value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers.¹ The addition of Lecithin and Polysorbate 80 to Tryptone Glucose Extract (TGE) Agar resulted in a medium that effectively neutralizes quaternary ammonium compounds while testing germicidal activity. Letheen Agar is a modification of TGE Agar with the addition of Lecithin and Polysorbate 80.

Letheen Broth Base was developed as a subculture medium for the neutralization of quaternary ammonium compounds in disinfectant testing. Quisno, Gibby, and Foter discovered that adding Lecithin and Polysorbate 80 to F.D.A. Broth resulted in a medium that neutralized high concentrations of quaternary ammonium salts.² The resulting medium, termed "Letheen" (a combination of Lecithin and Tween) was easy to prepare and clear in appearance, aiding to visual inspection for growth. Letheen Broth Base is recommended by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) for use with disinfectants containing cationic surface active materials.³

Letheen Broth Base is specified for use by the American Society for Testing Materials (ASTM) in Standard Test Method for Preservatives in Water-Containing Cosmetics.⁴ Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test.

Principles of the Procedure

Enzymatic Digest of Animal Tissue and Beef Extract provide nitrogen, carbon, vitamins, and minerals in Letheen Broth Base. Sodium Chloride maintains the osmotic balance. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with Lecithin, ethanol.^{2,5-7}

Formula / Liter

Enzymatic Digest of Animal Tissue.....	10 g
Beef Extract	5 g
Sodium Chloride	5 g
Lecithin.....	0.7 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / Liter

Polysorbate 80, 5 g

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 20.7 g of the medium and 5 g of Polysorbate 80 in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium amber and clear.

Expected Cultural Response: Cultural response in Lethen Broth Base at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Enterococcus faecalis</i> ATCC® 29212	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Staphylococcus epidermidis</i> ATCC® 12228	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Lethen Broth Base is used in a variety of procedures. Consult appropriate references for complete information.^{3,4}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Lethen Broth Base	Code No.	7105A	500 g
		7105B	2 kg
		7105C	10 kg

References

1. Weber, G. R., and L. A. Black. 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. **24**:134-139.
2. Quisno, R., I. W. Gibby, and M. J. Foter. 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118**:320-323.
3. Association of Official Analytical Chemists. 1995. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, D.C.
4. American Society for Testing Materials. 1991. Standard test method for preservatives in water-containing cosmetics, E 640-78. Annual Book of ASTM Standards, Philadelphia, PA.
5. Erlandson, A. L., Jr., and C. A. Lawrence. 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science. **118**:274-276.
6. Brummer, B. 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. Appl. Environ. Microbiol. **32**:80-84.
7. Favero (chm.). 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LETTEEN BROTH BASE, MODIFIED (7496)

Intended Use

Letheen Broth Base, Modified is used with Polysorbate 80 for the recovery of microorganisms from cosmetics.

Product Summary and Explanation

In 1948, Weber and Black described the value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers.¹ The addition of Lecithin and Polysorbate 80 to Tryptone Glucose Extract (TGE) Agar resulted in a medium that effectively neutralizes quaternary ammonium compounds in testing of germicidal activity. Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test.

Letheen Broth Base, Modified is based on the formula described in FDA Bacteriological Analytical Manual, and a modification of Letheen Broth Base.² Letheen Broth Base, Modified is recommended by the FDA for use in the microbiological testing of cosmetics.³

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen and carbon required for good growth of a wide variety of bacteria and fungi. The nitrogen level is increased in Letheen Broth Base, Modified to provide better growth. Yeast Extract provides vitamins and cofactors in this medium. Sodium Bisulfite, Polysorbate 80, and Lecithin (present in Letheen Broth Base) neutralizes quaternary ammonium compounds. Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with Lecithin, ethanol.^{4,5,6,7} These preservatives are commonly used in the cosmetic industry.

Formula / Liter

Letheen Broth Base	20.7 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	10 g
Yeast Extract.....	2 g
Sodium Bisulfite	0.1 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / Liter

Polysorbate 80, 5 g

Precautions

1. For Laboratory Use.
2. HARMFUL. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 37.8 g of the medium and 5 g of Polysorbate 80 in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is orange to amber, clear to slightly hazy with no moderate precipitate.

Expected Cultural Response: Cultural response in Lethen Broth Base, Modified at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Enterococcus faecalis</i> ATCC® 29212	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure³

1. Prepare and dilute samples in Lethen Broth Base, Modified in accordance with established guidelines. Proceed with procedure using Lethen Agar Base, Modified, Product No. 7495.
2. Using the spread plate technique, inoculate in duplicate 0.1 mL of the diluted samples onto Lethen Agar Base, Modified, Potato Dextrose Agar containing Chlortetracycline, Baird Parker Agar, Anaerobic Agar, and a second set of Lethen Agar Base, Modified plates.
3. Incubate one set of Lethen Agar Base, Modified at 30°C for 48 hours and the other set at 35°C under anaerobic conditions for 2 - 4 days. Incubate the Potato Dextrose Agar plates at 30°C for 7 days and the Baird Parker Agar plates, if inoculated, at 35°C for 48 hours.
4. Incubate diluted samples from Step 1 at 35°C for 7 days. Subculture enriched samples onto Lethen Agar Base, Modified only if there is no growth on the primary Lethen Agar Base, Modified.

Results

Refer to appropriate references for results, or in Lethen Agar Base, Modified, Product No. 7495, Product Information Sheet.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Lethen Broth Base, Modified	Code No.	7496A	500 g
		7496B	2 kg
		7496C	10 kg

References

1. **Weber, G. R., and L. A. Black.** 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. **24**:134-139.
2. **Tomlinson, L. (ed.).** 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
3. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1992. In Tomlinson, L.A. (ed.). FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
4. **Quisno, R., I. W. Gibby, and M. J. Foter.** 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118**:320-323.
5. **Erlanson, A. L., Jr., and C. A. Lawrence.** 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science. **118**:274-276.
6. **Brunner, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. Appl. Environ. Microbiol. **32**:80-84.
7. **Favero (chm.).** 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LISTERIA ENRICHMENT BROTH (7398)

Intended Use

Listeria Enrichment Broth is used for selective enrichment of *Listeria* spp.

Product Summary and Explanation

Listeria monocytogenes, first described in 1926 by Murray, Webb, and Swann,¹ is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.² Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey frankfurters,⁴ coleslaw, pasteurized milk, Mexican style cheese, and pate'. *Listeria* spp. are ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage and river water.⁵

Listeria Enrichment Broth is based on the formula developed by Lovett et al.⁶ in which Tryptic Soy Broth is supplemented with Yeast Extract for optimum growth. *Listeria* spp. grow over a pH range of 5.0 - 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, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Soybean Meal, and Yeast Extract provides nitrogen, vitamins, and minerals in Listeria Enrichment Broth. Dextrose is a carbohydrate source. Sodium Chloride maintains osmotic balance of the medium. Dipotassium Phosphate is a buffering agent. Nalidixic Acid inhibits growth of gram-negative organisms. Acriflavin inhibits gram-positive bacteria. Cycloheximide is used to inhibit growth of saprophytic fungi.

Formula / Liter

Enzymatic Digest of Casein	17 g
Enzymatic Digest of Soybean Meal	3 g
Yeast Extract.....	6 g
Dextrose.....	2.5 g
Sodium Chloride	5 g
Dipotassium Phosphate	2.5 g
Cyclohexamide	0.05 g
Acriflavin	0.015 g
Nalidixic Acid.....	0.04 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. TOXIC. Harmful by inhalation and if swallowed. Possible risk to unborn child.

Directions

1. Dissolve 36.1 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is orange amber with green opalescent top.

Expected Cultural Response: Cultural response in *Listeria* Enrichment Broth at 30°C after 24 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	good growth
<i>Listeria monocytogenes</i> ATCC® 15313	good growth
<i>Staphylococcus aureus</i> ATCC® 25923	suppressed at 24 hours; growth at 48 hours

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Use recommended laboratory procedures for isolating *Listeria* in food samples.

Results

Refer to appropriate references and procedures for results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. *Listeria* spp., other than *Listeria monocytogenes*, can grow on isolation media. An identification of *Listeria monocytogenes* must be confirmed through biochemical and serological testing.⁸

Packaging

Listeria Enrichment Broth	Code No.	7398A	500 g
		7398B	2 kg
		7398C	10 kg

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bact. **29**:407-439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
4. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
5. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
6. **Lovette, J., D. W. Frances, and J. M. Hunt.** 1987. *Listeria monocytogenes* In raw milk: detection, incidence and pathogenicity. J. Food Prot. **50**:188-192.
7. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LITTMAN AGAR (7173)

Intended Use

Littman Agar is used for the isolation and cultivation of fungi.

Product Summary and Explanation

In 1947, Littman Agar was described as suitable for growth of pathogenic fungi at a neutral pH.¹ Littman Agar is a selective medium for the primary isolation of fungi. Littman demonstrated this medium as valuable for culturing dermatophytes. Molds and yeasts form nonspreading, discrete colonies easily isolated in pure culture. Littman suggested using this medium for estimating normal fungal flora of sputum, feces, and other human specimens. Littman Agar can be used for single cell isolation of fungi and plate counts of viable saprophytic fungi in foodstuffs and air.

Littman compared Littman Agar with Sabouraud Dextrose Agar using a large variety of pathogenic and saprophytic fungi.² Three times as many fungi from feces, sputum, skin scrapings, and hair grew on Littman Agar as compared with Sabouraud Dextrose Agar. Four times as many pathogenic dermatophytes grew on Littman Agar as compared with Sabouraud Dextrose Agar.

Principles of the Procedure

Enzymatic Digest of Gelatin provides nitrogen, amino acids, vitamins, and carbon required for organism growth in Littman Agar. Dextrose is included as an energy source. Oxgall restricts the spreading of fungus colonies. Crystal Violet and Streptomycin are selective bacteriostatic agents. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Oxgall.....	15 g
Dextrose.....	10 g
Crystal Violet.....	0.01 g
Agar	16 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Streptomycin, 30 mcg

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 51 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 – 50°C and add 30 mcg of Streptomycin per mL of medium.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light grey-beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and bluish-purple to blue.

Expected Cultural Response: Cultural response on Littman Agar with the addition of streptomycin within 7 days at 25 - 30°C incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi. Do not use Littman Agar for the isolation of microorganisms susceptible to streptomycin.
3. Although culture techniques are primary in the identification of etiological agents of mycotic infections, they are not absolute. Often identification must be accomplished by using one or more of the following techniques: direct microscopic examination of the specimen, biochemical determination, or serological procedures.

Packaging

Littman Agar	Code No.	7173A	500 g
		7173B	2 kg
		7173C	10 kg

References

1. **Littman, M. L.** 1947. Culture medium for primary isolation of fungi. *Science*. **106**:109-111.
2. **Littman, M. L.** 1948. Growth of pathogenic fungi on a culture medium. *Am. J. Clin. Pathol.* **18**:409-420.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LOWENSTEIN - JENSEN MEDIUM (7245)

Intended Use

Lowenstein - Jensen Medium is used with fresh eggs and glycerol for the isolation and differentiation of *Mycobacterium* spp.

Product Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.¹ Non-tuberculous mycobacteria infections have also increased since 1985.² At least 25 species of mycobacteria are associated with human disease and produce usually slowly developing, destructive granulomas that may undergo necrosis with ulceration or cavitation.²

The use of egg-based media for primary isolation of mycobacteria have two significant advantages. First, egg-based media support a wide variety of mycobacteria. Second, growth of mycobacteria on egg media can be used for niacin testing. Liquification of Lowenstein-Jensen Medium can occur if contaminated with proteolytic organisms.

Lowenstein-Jensen Medium is a modification of Lowenstein Medium³, modified by Jensen.⁴ Jensen modified the medium by alternating the citrate and phosphate contents, eliminating congo red dye, and increasing malachite green concentration.⁵ Lowenstein-Jensen Medium is commonly used in the clinical laboratory to isolate acid fast organisms from sterile and nonsterile sources.⁶

Principles of the Procedure

L-Asparagine and Potato Flour are sources of nitrogen and vitamins in Lowenstein-Jensen Medium. Monopotassium Phosphate and Magnesium Sulfate enhance organism growth and act as buffers. Glycerol and the Egg Suspension 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. Sodium Citrate and Malachite Green are selective agents to prevent growth of most contaminants and allow early growth of mycobacteria.

Formula / Liter

L-Asparagine.....	3.6 g
Monopotassium Phosphate	2.5 g
Magnesium Sulfate	0.24 g
Sodium Citrate	0.6 g
Malachite Green.....	0.4 g
Potato Flour	30 g

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Glycerol, 12 mL
Egg Suspension, 1000 mL

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 37.3 g of the medium in 600 mL of purified water containing 12 mL of glycerol.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 1000 mL of a uniform suspension of fresh eggs under aseptic conditions. Avoid whipping air into suspension during the collection and mixing.
5. Aseptically mix the 1000 mL of egg suspension with 600 mL of the sterile Lowenstein-Jensen Medium cooled to 50 - 60°C, avoiding air bubbles.
6. Dispense the finished medium into sterile screw-cap test tubes. Place the tubes in a slanted position and heat at 85°C for 45 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and blue-green.

Prepared Appearance: Prepared medium with egg suspension is greenish-blue and opaque.

Expected Cultural Response: Cultural response on Lowenstein-Jensen Medium at 35°C after 2 – 3 weeks incubation.

Microorganism	Response
<i>Mycobacterium fortuitum</i> Group IV ATCC® 6841	growth
<i>Mycobacterium intracellulare</i> Group III ATCC® 13950	growth, may be inhibited on Selective L-J
<i>Mycobacterium kansasii</i> Group I ATCC® 12478	growth
<i>Mycobacterium scrofulaceum</i> Group II ATCC® 19981	growth, may be inhibited on Selective L-J
<i>Mycobacterium tuberculosis</i> H37Ra ATCC® 25177	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to specific procedures for a complete discussion of the isolation and identification of *Mycobacterium* spp.

Results

Observe for colonies that may or may not be pigmented. Colony morphology depends on the species isolated.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Mycobacterium* spp.
2. Negative culture results do not rule out an active mycobacterial infection.

Packaging

Lowenstein - Jensen Medium	Code No.	7245A	500 g
		7245B	2 kg
		7245C	10 kg

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria: molecular genetic insights. *Clinical Microbiology Reviews*. **8**:496-514.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Lowenstein, E.** 1931. Die Zuchtung der Tuberkelbazillen aus dem stramenden Blute. *Zentralb. Bakteriologie Parasitenkd. infektionskr. hyg. Abt. I orig.*, **120**:127.
4. **Jensen, K. A.** 1932. Rinzuchtung und Typenbestimmung von Tuberkelbazillanten. *Zentralb. Bakteriologie Parasitenkd. infektionskr. hyg. Abt. I Orig.*, **125**:222.
5. **MacFaddin, J. F.** 1985. *Media for isolation-cultivation-identification-maintenance of medical bacteria*, vol. 1. Williams & Wilkins, Baltimore, MD.
6. **Isenberg, H. D. (ed.).** 1992. *Clinical microbiology procedures handbook*, vol. 1 American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LPM AGAR (7424)

Intended Use

LPM Agar is used with moxalactam for the selective isolation of *Listeria* spp. from foods.

Product Summary and Explanation

Listeria monocytogenes, described in 1926 by Murray, Webb and Swann, is an extensive problem in public health and food industries.¹ This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.² Epidemiological evidence from outbreaks of listeriosis indicate the principle route of transmission is via consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey frankfurters,⁴ coleslaw, pasteurized milk, Mexican style cheese, and pate'. *Listeria* spp. are ubiquitous in nature, present in a wide range of unprocessed foods as well as soil, sewage, and river water.⁵

LPM Agar is a modification of McBride Listeria Agar, developed by Lee and McClain to recover low numbers of *Listeria monocytogenes* from samples with profusely mixed microflora.⁶ LPM Agar is recommended for testing food and dairy samples and clinical specimens for *Listeria* spp. *Listeria* spp. grow over a pH range of 5.0 - 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, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* spp. is based on successful isolation, biochemical characterization, and serological confirmation.

Principles of the Procedure

LPM Agar contains Enzymatic Digest of Casein, Enzymatic Digest of Soybean Meal, and Beef Extract to provide nitrogen, carbon, amino acids, and vitamins. Sodium Chloride maintains the osmotic balance of the medium. Lithium Chloride and Phenylethanol are incorporated to aid in suppression of Gram-positive and Gram-negative contaminants. Glycine Anhydride is used for improved recovery of *Listeria* spp. Agar is the solidifying agent. Selectivity is increased by adding Moxalactam to inhibit staphylococci, *Pseudomonas* spp., and *Proteus* spp.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Soybean Meal	5 g
Beef Extract	3 g
Sodium Chloride	5 g
Lithium Chloride	5 g
Glycine Anhydride	10 g
Phenylethanol	2.5 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobics

Moxalactam (10 mL), 2 mg/mL

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system and skin. May cause harm to unborn child.

Directions

1. Suspend 50.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and aseptically add 10 mL of a filter sterilized aqueous solution of moxalactam (2 mg/mL).
5. Dispense into sterile petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and light to medium amber.

Expected Cultural Response: Cultural response on LPM Agar supplemented with moxalactam at 35°C after 24 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	growth
<i>Listeria monocytogenes</i> ATCC® 15313	growth
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Clinical specimens obtained from nonsterile sites, foods, and environment samples should be selectively enriched for *Listeria* spp. before being plated. Refer to appropriate references for the isolation and identification from clinical specimens.⁷ To isolate *Listeria* spp. from milk, milk products, and food samples, refer to appropriate references.⁸⁻¹⁰

Results

Observe colonies under oblique transmitted light. *Listeria* colonies display a grey to blue color with a ground glass appearance.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. An identification of *L. monocytogenes* must be confirmed through biochemical and serological testing.⁷

Packaging

LPM Agar	Code No.	7424A	500 g
		7424B	2 kg
		7424C	10 kg

References

1. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bacteriol. **29**:407-439.
2. Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. Bremer, P. J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
4. Gaud, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
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7. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. Vanderzant, C., and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
9. Hitchins, A. D. 1992. *Listeria monocytogenes*, p. 141-151. FDA Bacteriological analytical manual, 7th ed. AOAC International, Arlington, VA.
10. Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig. 1993. Pathogens in milk and milk products. In R. T. Marshall (ed.). Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LURIA AGAR (7213) (MILLER'S LB AGAR)

Intended Use

Luria Agar (Miller's LB Agar) is used in molecular genetic studies.

Product Summary and Explanation

Luria Agar, a nutritionally rich medium designed for growth of pure cultures of recombinant strains, is based on Luria Broth Agar described by Miller.¹ *E. coli* is grown to late log phase in LB Medium. Some plasmid vectors replicate to a high copy number 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 prevent replication of the bacterial chromosome.²

Luria Agar contains 10 g/L of sodium chloride, different from the levels in Lennox and Miller formulations of LB Agar.¹⁻³ This allows the researcher to select the optimal salt concentration for a specific strain. The medium may be aseptically supplemented with glucose.

Principles of the Procedure

The nitrogen, amino acids, and carbon sources are provided by Enzymatic Digest of Casein. Vitamins and certain trace elements are supplied by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Yeast Extract.....	5 g
Sodium Chloride	10 g
Agar	12 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 37 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and yellow beige.

Prepared Appearance: Prepared medium is trace to slightly hazy.

Expected Cultural Response: Cultural response on Luria Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Escherichia coli</i> ATCC® 25922	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.^{1,2}

Results

After sufficient incubation the medium should show growth as evidenced by formation of isolate colonies and/or confluent lawn of growth.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Luria Agar (Miller's LB Agar)	Code No.	7213A	500 g
		7213B	2 kg
		7213C	10 kg

References

1. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
3. **Lennox E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology. **1**:190-206.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

LURIA BROTH (7279) (MILLER'S LB BROTH)

Intended Use

Luria Broth (Miller's LB Broth) is used in molecular genetic studies.

Product Summary and Explanation

Luria Broth is based on the Luria Broth formula described by Miller.¹ This medium is used for the growth and maintenance of *Escherichia coli* strains used in molecular microbiology procedures. Luria Broth Base is a nutritionally rich medium designed for growth of pure cultures of recombinant strains.¹ *E. coli* is grown to late log phase in LB Medium. Some plasmid vectors replicate to high copy numbers without selective amplification. Some vectors do not replicate so freely, and need to be selectively amplified. Chloramphenicol can be added to inhibit host synthesis and prevent replication of the bacterial chromosome.²

Luria Broth contains 10 g/L of sodium chloride. The medium may be aseptically supplemented with glucose.

Principles of the Procedure

The nitrogen, amino acids, and carbon sources are provided by Enzymatic Digest of Casein. Vitamins and certain trace elements are supplied by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride.

Formula / Liter

Enzymatic Digest of Casein 10 g

Yeast Extract..... 5 g

Sodium Chloride 10 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 25 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear and yellow to gold.

Expected Cultural Response: Cultural response in Luria Broth at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Escherichia coli</i> ATCC® 25922	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.^{1,2}

Results

After sufficient incubation growth is evident by the appearance of turbidity.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Luria Broth (Miller's LB Broth)	Code No.	7279A	500 g
		7279B	2 kg
		7279C	10 kg

References

1. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Technical Information

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LYSINE IRON AGAR (7211)

Intended Use

Lysine Iron Agar is used for the differentiation of microorganisms on the basis of lysine decarboxylase and hydrogen sulfide production.

Product Summary and Explanation

Lysine Iron Agar is prepared according to the formulation of Edwards and Fife, who developed the medium to detect *Salmonella arizonae*.¹ *S. arizonae* ferments lactose rapidly, and the authors found expected H₂S production on Triple Sugar Iron Agar was suppressed. Detection of *S. arizonae* is important because it has been implicated in food borne infections. By eliminating lactose and incorporating lysine, Edwards and Fife devised a medium differentiating enteric bacilli based on their ability to decarboxylate or deaminate lysine and produce abundant hydrogen sulfide. This medium is recommended for detecting rapid lactose-fermenting *S. arizonae*.

Lysine Iron Agar is specified in standard methods for *Salmonella* testing.²⁻⁶

Principles of the Procedure

Enzymatic Digest of Gelatin provides carbon, nitrogen, and amino acids required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth, and additional sources of nitrogen and carbon. Dextrose is an energy source. L-Lysine is the substrate used to detect lysine decarboxylase and lysine deaminase enzymes. Ferric Ammonium Citrate is an indicator of hydrogen sulfide production. Sodium Thiosulfate is added as a source of inorganic sulfur. Bromcresol Purple, a pH indicator, is yellow at or below pH 5.2 and purple at or above pH 6.8. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	5 g
Yeast Extract.....	3 g
Dextrose.....	1 g
L-Lysine.....	10 g
Ferric Ammonium Citrate.....	0.5 g
Sodium Thiosulfate	0.04 g
Bromcresol Purple	0.02 g
Agar	13.5 g

Final pH: 6.7 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 33 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into test tubes and autoclave at 121°C for 15 minutes.
4. After autoclaving, allow medium to solidify in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and gray to grayish beige.

Prepared Appearance: Prepared medium is red-purple and trace to slightly hazy.

Expected Cultural Response: Cultural response on Lysine Iron Agar at 35°C after 18 –48 hours incubation.

Microorganism	Response	Reactions		
		Slant	Butt	H ₂ S
<i>Citrobacter freundii</i> ATCC® 8090	growth	K	A	+
<i>Escherichia coli</i> ATCC® 25922	growth	K	K	---
<i>Proteus mirabilis</i> ATCC® 12453	growth	R	A	---
<i>Salmonella typhimurium</i> ATCC® 14028	growth	K	K	+

The organisms listed are the minimum that should be used for quality control testing.

KEY: K, alkaline, R, red (oxidative deamination), A, acid +, H₂S produced, ---, H₂S not produced

Test Procedure

1. Inoculate medium by stabbing base of tube butt and streaking slant with a needle.
2. Loosely cap the tube to ensure aerobic conditions. Incubate at 35°C for 18 - 48 hours.
3. Examine at 18 – 24 and 40 – 48 hours for growth and color changes in tube butt and slant, and for blackening at the apex of slant.

Results

- A positive lysine decarboxylase reaction is purple (alkaline) butt, purple slant. A negative reaction is yellow (acid) butt, purple (alkaline) slant.
- A positive lysine deaminase reaction is a red slant. A negative reaction is a purple slant. (*Proteus* spp. and *Providencia* spp. produce a red slant over a yellow [acid] butt.)
- A positive hydrogen sulfide reaction is blackened medium at the apex of the slant.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. *Salmonella paratyphi* A, unlike other *Salmonella* spp., does not produce lysine decarboxylase resulting in an alkaline slant and an acid butt.
2. H₂S-producing *Proteus* spp. do not blacken the medium.^{2,7} It is suggested that Lysine Iron Agar be used in conjunction with Triple Sugar Iron Agar or other media to confirm differentiation.
3. The reaction of *Morganella morganii* may be variable after 23 hours incubation and may require longer incubation.⁷

Packaging

Lysine Iron Agar	Code No.	7211A	500 g
		7211B	2 kg
		7211C	10 kg

References

1. **Edwards, P. R., and M. A. Fife.** 1961. Lysine-iron agar in the detection of *Arizona* cultures. Appl. Microbiol. **9**:478.
2. **MacFaddin, J. F.** Media for isolation-cultivation-identification-maintenance of medial bacteria, vol 1. Williams & Wilkins, Baltimore, MD.
3. **Vanderzant, C. and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1992. Pathogens in milk and milk products, p. 103-212. In R. T. Marshall, (eds.). Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.
6. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. In Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, M.D.
7. **Finegold, S. M., and W. J. Martin.** 1982. Bailey and Scott's diagnostic microbiology, 6th ed. The CV Mosby Company, St. Louis, MO.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

M-BROTH (7296)

Intended Use

M-Broth is used for the cultivation of *Salmonella* spp.

Product Summary and Explanation

M-Broth is prepared according to the formula of Sperber and Diebel,¹ and contains the nutrients necessary for good growth and flagella development of *Salmonella*. M-Broth is used for cultivating *Salmonella* in foods and feeds by the accelerated enrichment serology (ES) procedure. M-Broth conforms to standard procedures recommended in food testing and monoclonal and polyclonal enzyme immunoassays (EIA).^{2,3}

Principles of the Procedure

Enzymatic Digest of Casein is the nitrogen source in M-Broth. Yeast Extract is a source of B-complex vitamins. D-Mannose and Sodium Citrate are the 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 / Liter

Enzymatic Digest of Casein	12.5 g
Yeast Extract.....	5 g
D-Mannose	2 g
Sodium Citrate	5 g
Sodium Chloride	5 g
Potassium Phosphate	5 g
Magnesium Chloride	0.14 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Polysorbate 80	0.75 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 36.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous with small lumps and light beige.

Prepared Appearance: Prepared medium is light amber and clear to trace hazy.

Expected Cultural Response: Cultural response in M-Broth at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Salmonella arizonae</i> ATCC® 13314	good growth
<i>Salmonella choleraesuis</i> ATCC® 13076	good growth
<i>Salmonella typhimurium</i> ATCC® 14028	good growth
<i>Salmonella typhi</i> ATCC® 19430	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Prepare a 10% suspension of the test sample in Lactose Broth. Incubate at $35 \pm 2^\circ\text{C}$ for 18 - 24 hours.
2. Transfer 1 mL of the above pre-enrichment culture to 9 mL of Selenite Cystine Broth, and 1 mL to 9 mL of Tetrathionate Broth. Incubate both enrichment media at $35 \pm 2^\circ\text{C}$ 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 $35 \pm 2^\circ\text{C}$ for 6 - 8 hours.
4. Prepare a formalin-salt solution by adding 4.2 grams of NaCl and 3 mL of formalin to 100 mL of distilled 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 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 of 0.85% NaCl solution to the other tube. Shake the tubes gently. Incubate in a 50°C waterbath for 1 ½ hours.

An alternative testing procedure can be found in AOAC International³ for screening procedures using enzyme immunoassay or DNA hybridization to detect Salmonella antigens in test samples.

Results

Agglutination in the Kahn tube containing salmonella antiserum indicates the presence of *Salmonella*. agglutination in the Kahn tube containing 0.8% 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.

Storage

Store the sealed bottle containing the dehydrated medium at $2 - 8^\circ\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

M-Broth	Code No.	7296A	500 g
		7296B	2 kg
		7296C	10 kg

References

1. **Sperber, W. H., and R. H. Deibel.** 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. Appl Microbiol. **17**:533-539.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Technical Information

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m-ENTEROCOCCUS AGAR (7544)

Intended Use

m-Enterococcus Agar is used for the selective isolation and enumeration of enterococci by membrane filtration.

Product Summary and Explanation

m-Enterococcus Agar was first described by Slanetz et al. for the enumeration of enterococci by the membrane filtration technique.¹ In 1957, Slanetz and Bartley modified this medium by adding triphenyltetrazolium chloride (TTC).² 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 is simple to perform, does not require confirmation, and permits a direct count of enterococci in 48 hours. m-Enterococcus Agar is also referred to as m-Azide Agar.

The enterococcus group are fecal streptococci and include *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. avium*.³ 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.³ The presence of enterococci is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.³ m-Enterococcus Agar is used in standard methods for the detection of fecal streptococci using the membrane filtration technique.³

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal provides the nitrogen, minerals, and amino acids in m-Enterococcus Agar. Yeast Extract is the vitamin source and Dextrose supplies carbon. Dipotassium Phosphate acts as a buffer. Sodium Azide is the selective agent used 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 insoluble formazan inside the bacterial cell, resulting in the production of red colonies.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5 g
Yeast Extract.....	5 g
Dextrose.....	2 g
Dipotassium Phosphate.....	4 g
Sodium Azide	0.4 g
2,3,5-Triphenyl Tetrazolium Chloride.....	0.1 g
Agar	10 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful by inhalation and if swallowed. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE. Cool to 45 - 50°C and dispense.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light pinkish-beige.

Prepared Appearance: Prepared medium is light to medium pink-beige, and clear to slightly hazy.

Expected Cultural Response: Cultural response on m-Enterococcus Agar at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 19433	growth	dark red colonies
<i>Enterococcus faecalis</i> ATCC® 29212	growth	dark red colonies
<i>Enterococcus faecalis</i> ATCC® 33186	growth	dark red colonies
<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Staphylococcus aureus</i> ATCC® 25923	Inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedures

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in standard methods or by laboratory policy.³
2. Choose a sample size resulting in 20 - 60 colonies.
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 steak plate method.
2. Incubate plates at 35 ± 2°C for 24 - 48 hours.

Results³

Count all light and dark red colonies as enterococci. Count colonies using a fluorescent lamp and a magnifying lens.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

m-Enterococcus Agar	Code No.	7544A	500 g
		7544B	2 kg
		7544C	10 kg

References

1. **Slanetz, Bent, and Bartley.** 1955. Public Health Rep. **70**:67.
2. **Slanetz, and Bartley.** 1957. J. Bacteriol. **74**:591.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

m-FC AGAR (7397)

Intended Use

m-FC Agar is used with rosolic acid for the detection and enumeration of fecal coliforms by membrane filtration.

Product Summary and Explanation

Geldreich et al. formulated a medium to enumerate fecal coliforms (FC) using the membrane filter (m) technique without prior enrichment.¹ Fecal coliforms, i.e., those found in feces of warm-blooded animals, are differentiated from environmental coliforms by their ability to grow at $44.5 \pm 0.5^\circ\text{C}$.²

Many standard method membrane filtration procedures recommend m-FC media for testing water. The American Public Health Association (APHA) specified m-FC media and incubation at $44.5 \pm 0.5^\circ\text{C}$ in the fecal coliform procedure and other tests.^{2,3} The Association of Official Analytical Chemists (AOAC) specifies m-FC Agar for detecting total coliforms and fecal coliforms in foods.⁴ The US Environmental Protection Agency specified using m-FC media in fecal coliform methods for testing water by the direct MF method or the delayed-incubation MF methods.^{5,6}

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and minerals in m-FC Agar. Yeast Extract is a source of vitamins and trace elements. Sodium Chloride maintains the osmotic balance. Lactose serves as a carbohydrate source. Bile Salts inhibit growth of gram-positive bacteria. The differential indicator system combines Aniline Blue and Rosolic Acid which is added as a supplement. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	9 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Yeast Extract.....	6.5 g
Sodium Chloride	5 g
Lactose	12.5 g
Bile Salts	1.5 g
Aniline Blue	0.1 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

1% Rosolic Acid, 1 mL

Precaution

1. For Laboratory Use.

Directions

m-FC Agar

1. Suspend 5.2 g of the medium in 100 mL of purified water containing 1 mL of 1% rosolic acid in 0.2 N NaOH.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Cool to $45 - 50^\circ\text{C}$ and pour plates.
4. DO NOT AUTOCLAVE.

Rosolic Acid

1. Dissolve 1 g in 100 mL of 0.2 N NaOH to prepare a 1% solution.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared unsupplemented medium is cornflower blue and clear to slightly hazy. Appearances with Rosolic Acid is trace to slightly hazy to cranberry red.

Expected Cultural Response: Cultural response on m-FC Agar at 44.5°C after 22 - 24 hours incubation.

Microorganism	Response	Reactions w/ Rosolic Acid
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	grey colonies, clear, translucent
<i>Escherichia coli</i> ATCC® 11775	growth	blue colonies, may have blue ppt.
<i>Escherichia coli</i> ATCC® 25922	growth	blue colonies, may have blue ppt.
<i>Klebsiella pneumoniae</i> ATCC® 13883	growth	grey to greyish-blue colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	grey, clear, translucent
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Filter duplicate samples through separate membrane filters.
2. Transfer filters to surface of separate m-FC Agar plates.
3. Place each plate in a separate waterproof plastic bag. Submerge in waterbath set at 44.5 ± 0.5°C; incubate for 24 ± 2 hours.

Results

Colonies of fecal coliforms will be various shades of blue. Non-fecal coliforms are grey to cream-colored.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. A few non-fecal coliform colonies may be observed on m-FC Agar due to the selective action of the elevated temperature and the addition of rosolic acid. It may be useful to elevate the temperature to 45 ± 0.2°C to eliminate *Klebsiella* strains from the fecal coliform group.²

Packaging

m-FC Agar	Code No.	7397A	500 g
		7397B	2 kg
		7397C	10 kg

References

1. **Geldreich, E. E., H. F. Clark, C. B. Huff, and L. C. Best.** 1965. Fecal-coliform-organism medium for the membrane filter technique. J. Am. Water Works Assoc. 57:208-214.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Cowman, S., and R. Kelsey.** 1992. Bottled water, p. 1031-1036. In C. Vanderzant, and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Andrews, W.** 1995. Microbial methods, p. 17.1-17-119. In Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
5. **Bordner, R., and J. Winter (eds.).** 1978. Microbiological methods for monitoring the environment. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
6. **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, U. S. Environmental Protection Agency, Cincinnati, OH.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

m-FC BROTH (7396)

Intended Use

m-FC Broth is used with rosolic acid for the detection and enumeration of fecal coliforms by membrane filtration.

Product Summary and Explanation

Geldreich et al. formulated a medium to enumerate fecal coliforms (FC) using the membrane filter (m) technique without prior enrichment.¹ Fecal coliforms, i.e., those found in feces of warm-blooded animals, are differentiated from environmental coliforms their ability to grow at $44.5 \pm 0.5^{\circ}\text{C}$.²

Many standard method membrane filtration procedures recommend m-FC media for testing water. The American Public Health Association (APHA) specified m-FC media and incubation at $44.5 \pm 0.5^{\circ}\text{C}$ in several procedures.^{2,3} The US Environmental Protection Agency specified using m-FC media in fecal coliform methods for testing water by the direct MF method or the delayed-incubation MF methods.^{4,5}

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and minerals in m-FC Broth. Yeast Extract is a source of vitamins and trace elements. Sodium Chloride maintains the osmotic balance. Lactose serves as a carbohydrate source. Bile Salts inhibit growth of Gram-positive bacteria. The differential indicator system combines Aniline Blue and Rosolic Acid, which is added as a supplement.

Formula / Liter

Enzymatic Digest of Casein	9 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Yeast Extract.....	6.5 g
Sodium Chloride	5 g
Lactose	12.5 g
Bile Salts	1.5 g
Aniline Blue	0.1 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

1% Rosolic Acid, 1 mL

Precaution

1. For Laboratory Use.

Directions

m-FC Broth

1. Dissolve 3.7 g of the medium in 100 mL of purified water containing 1 mL of 1% rosolic acid in 0.2 N NaOH.
2. Heat with frequent agitation to boiling to completely dissolve the medium.
3. Cool to room temperature.

Rosolic Acid

1. Dissolve 1 g in 100 mL of 0.2 N NaOH to prepare a 1% solution.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and bluish beige.

Prepared Appearance: Prepared unsupplemented medium is cornflower blue and clear to slightly hazy. With Rosolic acid, medium is cranberry red.

Expected Cultural Response: Cultural response in m-FC Broth at 44.5°C after 22 - 24 hours incubation.

Microorganism	Response	Reactions w/ Rosolic Acid
<i>Escherichia coli</i> ATCC® 11775	growth	blue colonies, may have blue ppt.
<i>Escherichia coli</i> ATCC® 25922	growth	blue colonies, may have blue ppt.
<i>Salmonella typhimurium</i> ATCC® 14028	growth	colorless colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for procedures using m-FC Broth.

Results

Following incubation, examine membrane filters for presence of colored colonies. Blue colonies are counted as fecal coliforms. Other organisms, non-fecal coliforms, form grey to cream colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

m-FC Broth	Code No.	7396A	500 g
		7396B	2 kg
		7396C	10 kg

References

1. **Geldreich, E. E., H. F. Clark, C. B. Huff, and L. C. Best.** 1965. Fecal-coliform-organism medium for the membrane filter technique. J. Am. Water Works Assoc. 57:208-214.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Cowman, S., and R. Kelsey.** 1992. Bottled water, p. 1031-1036. In C. Vanderzant, and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Bordner, R., and J. Winter (eds.).** 1978. Microbiological methods for monitoring the environment. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
5. **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, U. S. Environmental Protection Agency, Cincinnati, OH.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

m-GREEN YEAST and FUNGI BROTH (7190)

Intended Use

m-Green Yeast and Fungi Broth is used for the detection of fungi in beverages.

Product Summary and Explanation

m-Green Yeast and Fungi Broth is a relatively more complex formula compared to other media used for isolation of fungi and yeast. This formulation is rich in nutrients which allows for excellent fungal growth. Bacterial growth is inhibited by an acid pH.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and amino acids in m-Green Yeast and Fungi Broth. Yeast Extract is the vitamin source. Dextrose is an energy source for metabolism of fungi. Potassium Phosphate is a buffering agent. Magnesium Sulfate, Thiamine, and Diastase (a mixture containing amylolytic (starch) enzymes) provide essential ions, minerals, and nutrients. Bromcresol Green is the pH indicator, facilitating visualization and counting of fungal colonies. The colonies are green due to diffusion of Bromcresol Green into the colonies. Acidic end products from colonies diffuse into the medium, further reducing the pH and causing the indicator to turn yellow (acid reaction) around the colonies.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Yeast Extract.....	9 g
Dextrose.....	50 g
Magnesium Sulfate	2.1 g
Potassium Phosphate	2 g
Diastase	0.05 g
Thiamine	0.05 g
Bromcresol Green.....	0.026 g

Final pH: 4.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 73 g of the medium in one liter of purified water.
2. Heat with frequent agitation to obtain solution.
3. Autoclave at 121°C for 10 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green-beige.

Prepared Appearance: Prepared medium is clear, dark green and may have a moderate ppt.

Expected Cultural Response: Cultural response in m-Green Yeast and Fungi Broth at 25 - 30°C after 2 - 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Saturate a sterile membrane filter pad in a sterile petri dish with 2.0 to 2.5 mL of m-Green Yeast and Fungi Broth.
2. Roll the membrane filter from the test sample onto the surface of the moistened pad. Avoid trapping air bubbles between the filter and the pad.
3. Incubate plates for up to 7 days at 25 - 30°C in an aerobic atmosphere.

Results

Count colonies appearing on the filter surface after incubation. Mold colonies generally appear green and filamentous, yeast colonies are green and opaque. Refer to appropriate references for complete information on isolation and identification of yeasts and molds.⁴

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

m-Green Yeast and Fungi Broth	Code No.	7190A	500 g
		7190B	2 kg
		7190C	10 kg

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

m-TEC AGAR (7421)

Intended Use

m-TEC Agar is used with urea for the isolation and enumeration of thermotolerant *Escherichia coli* from water using the membrane filtration technique.

Product Summary and Explanation

Escherichia coli is used as an indicator of fecal pollution in water. Several tests are available for enumerating *E. coli* based on its ability to grow at elevated temperatures and indole production.^{1,2} The membrane filter procedure is recognized in Standard Methods as an alternate test procedure.³ m-TEC is an abbreviation for membrane thermotolerant *E. coli*.

In 1981, Dufour et al. developed a simple and accurate membrane filter technique for rapid enumeration of *E. coli*.⁴ In this study, the researchers were able to quantitate *E. coli* on m-TEC Agar within 24 hours without requiring subculture and identification of isolates. Dufour et al. recovered *E. coli* from marine, estuarine, and fresh water samples.⁴

Principles of the Procedure

Enzymatic Digest of Animal Tissue provides nitrogen, carbon, and minerals in m-TEC Agar. Yeast Extract is a source of vitamins and trace elements. Lactose serves as a carbon source. Potassium Phosphate is a buffering agent. Sodium Lauryl Sulfate and Sodium Deoxycholate are selective agents against gram-positive bacteria. Bromcresol Purple and Bromphenol Red are pH indicators. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	5 g
Yeast Extract.....	3 g
Lactose	10 g
Sodium Chloride	7.5 g
Potassium Phosphate	4.3 g
Sodium Lauryl Sulfate	0.2 g
Sodium Deoxycholate	0.1 g
Bromcresol Purple	0.08 g
Bromphenol Red	0.08 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

m-TEC Agar

1. Suspend 45.3 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Dispense 4 – 5 mL amounts into 10 x 50 mm petri dishes, allow to solidify.

Urea Substrate

1. Combine 2 g urea and 10 mg phenol red in 100 mL purified water.
2. Adjust pH to 5.0 ± 0.3
3. Store at 2 - 8°C. Use within one week.

Note: Other methods may recommend an alternative pH.^{3,6} Prepare substrate according to recommended guidelines.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light grey-green beige.

Prepared Appearance: Prepared medium at 45 - 50°C is clear to trace hazy and dark purple.

Expected Cultural Response: Cultural response on m-TEC Agar at 44.5°C after 24 – 48 hours incubation.

Microorganism	Response	Reactions w/ Urease Substrate
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	---
<i>Escherichia coli</i> ATCC® 8739	growth	yellow colonies
<i>Escherichia coli</i> ATCC® 35150	growth	yellow colonies
<i>Escherichia coli</i> ATCC® 35218	growth	yellow colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Follow membrane filter procedure described in Standard Methods.¹
2. Incubate inoculated plates for 2 hours at 35°C to resuscitate injured cells.
3. Transfer plates to a 44.5 ± 0.5°C waterbath or incubator and incubate for 20 ± 2 hours.
4. Place a 50 mm absorbent pad into petri dish. Add approximately 2 mL of urea substrate to pad (pad should be saturated with urea substrate without any standing liquid in petri dish).
5. Transfer countable filters to pads saturated with urea substrate.
6. After 15 - 20 minutes, count all yellow to yellow-brown colonies with the aid of a stereoscopic microscope.

Results

Yellow to yellow-brown colonies (urease negative) may be presumptively identified as *E. coli*.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if medium has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may grow poorly or fail to grow on this medium.
2. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
3. The urease test is required to presumptively identify *E. coli*.
4. Choose a water sample size that will result in 20 - 80 colonies per filter. Higher counts may not provide accurate urease test results.
5. Do not trap air bubbles underneath the filter.

Packaging

m-TEC Agar	Code No.	7421A	500 g
		7421B	2 kg
		7421C	10 kg

References

1. **Mara, D. D.** 1973. A single medium for the rapid detection of *Escherichia coli* at 44°C. J. Hyg. **71**:783-785.
2. **Pugsley, A. P., L. J. Evison, and A. James.** 1973. A simple technique for the differentiation of *Escherichia coli* in water examination. Water RES. **7**:1431-1437.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Dufour, A. P., E. R. Strickland, and V. J. Cabelli.** 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. **41**:1152-1158.
5. **Dufour, A. P., and V. J. Cabelli.** 1975. Membrane filter procedure for enumerating the component genera of the coliform group in seawater. Appl. Microbiol. **29**:826-833.
6. **1996 Annual Book of ASTM Standards,** Water and Environmental Technology (PCN: 01-11-296-16). ASTM, West Conshohocken, PA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

m-TGE BROTH (7451)

Intended Use

m-TGE Broth is used for the determination of bacterial counts using membrane filtration.

Product Summary and Explanation

m-TGE is an abbreviation for membrane Tryptone Glucose Extract. In the 1930's, Bower and Hucker developed a medium for detecting bacteria in milk and other dairy products.¹ Prickett used a glucose agar containing tryptone to study thermophilic bacteria in milk.² This medium is known as Yeast Dextrose Agar. In 1948, the American Public Health Association (APHA) adopted Tryptone Glucose Extract Agar for use in testing milk and dairy products.³ Currently, APHA specifies Tryptone Glucose Extract Agar for the heterotrophic plate count procedure in testing bottled water.⁴

m-TGE Broth is a nonselective nutrient medium for the determination of bacterial counts by the membrane filtration method. This broth has the same formulation as Tryptone Glucose Extract Agar, except the agar has been omitted, and ingredients are at twice the concentration.

Principles of the Procedure

Enzymatic Digest of Casein and Beef Extract provide the nitrogen, minerals, vitamins, and amino acids in m-TGE Broth. Dextrose supplies carbon as an energy source.

Formula / Liter

Enzymatic Digest of Casein 10 g
Beef Extract 6 g
Dextrose..... 2 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 18 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light medium tan.

Prepared Appearance: Prepared medium is clear and amber.

Expected Cultural Response: Cultural response in m-TGE Broth at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Micrococcus luteus</i> ATCC® 9341	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedures

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in standard methods or by laboratory policy.³
2. Incubate the inoculated medium in a humid atmosphere at $35 \pm 2^\circ\text{C}$ for 18 - 24 hours incubation.

Results³

Count total colonies and record results.

Storage

Store sealed bottle containing dehydrated medium at $2 - 30^\circ\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

m-TGE Broth	Code No.	7451A	500 g
		7451B	2 kg
		7451C	10 kg

References

1. **Slanetz, Bent, and Bartley.** 1955. Public Health Rep. **70**:67.
2. **Slanetz, and Bartley.** 1957. J. Bacteriol. **74**:591.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

M17 BROTH BASE (7450)

Intended Use

M17 Broth Base is used for the isolation and cultivation of lactic streptococci from dairy products.

Product Summary and Explanation

Lactic streptococci are acid-producing bacteria. They are nutritionally fastidious and require complex culture media for optimum growth. In a synthetic medium, one study revealed all strains had an obligate requirement for at least six amino acids and three vitamins.¹ These homofermentative lactic streptococci produce large amounts of acid. In a culture medium without an adequate buffering system the pH decreases and adversely affects organism growth. Lowrie and Pearce developed M16 Medium but it lacked a strong buffering system.² Terzaghi and Sandine found M16 Medium demonstrated a rapid drop in pH with growth of lactic streptococcal growth.³ This decrease in pH can adversely affect colony size and phage plaque formation.³ Terzaghi and Sandine modified M16 Medium using disodium-β-glycerophosphate as a buffer and called it M17.³

Shankar and Davies found Disodium-β-glycerophosphate in M17 Broth suppressed *Lactobacillus bulgaricus* and selectively isolated *Streptococcus thermophilus* from yogurt.⁴ Similar results were achieved using M17 Broth solidified with agar.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide carbon, nitrogen, and amino acids used in M17 Broth Base. Yeast Extract supplies B-complex vitamins to stimulate bacterial growth. Ascorbic Acid stimulates growth of lactic streptococci. Magnesium Sulfate provides essential ions. Disodium-β-glycerophosphate buffers the medium as acid is produced from fermentation of lactose from dairy products.

Formula / Liter

Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Beef Extract	3 g
Yeast Extract.....	2.5 g
Ascorbic Acid	0.5 g
Magnesium Sulfate	0.25 g
Final pH: 7.0 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Disodium-β-glycerophosphate 19 g

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 16.2 g of the medium and 19 g of disodium-β-glycerophosphate in one liter of purified water.
2. Mix with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and tan.

Prepared Appearance: Prepared medium is clear with no to slight precipitate.

Expected Cultural Response: Cultural response in M17 Broth Base at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Lactococcus lactis</i> ATCC® 19257	growth
<i>Micrococcus</i> spp. ATCC® 51819	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

M17 Broth Base	Code No.	7450A	500 g
		7450B	2 kg
		7450C	10 kg

References

1. **Reiter, B., and J. D. Oram.** 1962. Nutritional studies on cheese starters. I. Vitamin and amino acid requirements of single strain starters. *J. Dairy Res.* **29**:63-77.
1. **Lowrie and Pearce.** 1971. *J. Dairy Sci. Technol.* **6**:166.
2. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. *Appl. Microbiol.* **29**:807-813.
3. **Shankar, P. A., and F. L. Davies.** 1977. A note on the suppression of *Lactobacillus bulgaricus* in media containing β -glycerophosphate and application of such media to selective isolation of *Streptococcus thermophilus* from yogurt. *J. Soc. Dairy Tech.* **30**:28-30.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MacCONKEY AGAR (7102)

Intended Use

MacConkey Agar is used for the isolation and differentiation of Gram-negative enteric bacilli.

Product Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.¹ The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula modifications improved differential reactions between enteric pathogens and coliforms.

MacConkey Agar is recommended for the detection and isolation of gram-negative organisms from clinical,² dairy,³ food,^{4,5} water,⁶ pharmaceutical,⁷ and industrial⁸ sources.

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar. Lactose is the fermentable carbohydrate. During Lactose fermentation a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation. Bile Salts Mixture and Crystal Violet are the selective agents, inhibiting gram-positive cocci and allowing Gram-negative organisms to grow. Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Lactose	10 g
Bile Salts Mixture	1.5 g
Sodium Chloride	5 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.001 g
Agar	13.5 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system and skin.

Directions

1. Suspend 50 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and dispense into sterile petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pink-beige.

Prepared Appearance: Prepared medium is red-purple and slightly opalescent.

Expected Cultural Response: Cultural response on MacConkey Agar at 37°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions	Bile ppt
<i>Enterococcus faecalis</i> ATCC® 29212	marked to complete inhibition	--	-
<i>Escherichia coli</i> ATCC® 25922	growth	pink colonies	+
<i>Proteus mirabilis</i> ATCC® 12453	growth with partial inhibition of swarming	colorless colonies	-
<i>Salmonella typhimurium</i> ATCC® 14028	growth	colorless colonies	-

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references using MacConkey Agar for the isolation and identification of enteric organisms.

Results

Lactose-fermenting organisms grow as pink to brick red colonies with or without a zone of precipitated bile. Non-lactose fermenting organisms grow as colorless or clear colonies.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Although MacConkey Agar is a selective medium primarily for Gram-negative enteric bacilli, biochemical and serological testing using pure cultures are recommended for complete identification.
3. Incubation of MacConkey Agar plates under increased CO₂ has been reported to reduce growth and recovery of a number of strains of Gram-negative bacilli.⁹

Packaging

MacConkey Agar	Code No.	7102A	500 g
		7102B	2 kg
		7102C	10 kg

References

1. **MacConkey, A.** 1905. Lactose-fermenting bacteria in feces. J. Hyg. 5:333-379.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
6. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
7. **United States Pharmacopeial Convention, Inc.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
8. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
9. **Mazura-Reetz, G. T. Neblett, and J. M. Galperin.** 1979. MacConkey Agar: CO₂ vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MacCONKEY AGAR w/o CRYSTAL VIOLET (7236)

Intended Use

MacConkey Agar w/o Crystal Violet is used for the isolation and differentiation of gram-negative enteric bacilli.

Product Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.¹ The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula changes improved differential reactions between enteric pathogens and coliforms.

MacConkey Agar w/o Crystal Violet is a differential medium and less selective than MacConkey Agar. The lack of Crystal Violet permits growth of enterococci, staphylococci, and *Mycobacterium* spp.

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar w/o Crystal Violet. Lactose is the fermentable carbohydrate. During Lactose fermentation, a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation develops. Bile Salts Mixture is the selective agent. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Lactose	10 g
Bile Salts Mixture	5 g
Sodium Chloride	5 g
Neutral Red.....	0.05 g
Agar	12 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light pink-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and red-orange.

Expected Cultural Response: Cultural response on MacConkey Agar w/o Crystal Violet at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	partial inhibition	pink to red
<i>Escherichia coli</i> ATCC® 25922	growth	bright pink colonies
<i>Proteus mirabilis</i> ATCC® 12453	growth with partial inhibition of swarming	colorless colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	colorless colonies
<i>Staphylococcus aureus</i> ATCC® 25923	growth	pink

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references using MacConkey Agar w/o Crystal Violet for the isolation and identification of enteric organisms.²

Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or without a zone of precipitated bile. Non-lactose fermenting organisms grow as colorless or clear colonies. Swarming by *Proteus* spp. is reduced.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Although MacConkey Agar w/o Crystal Violet is a selective medium, it is less inhibitory than MacConkey Agar, allowing Gram-positive organisms to grow. Biochemical and serological testing using pure cultures are recommended for complete identification.
3. Incubation of MacConkey Agar w/o Crystal Violet under increased CO₂ has been reported to reduce growth and recovery of certain Gram-negative bacilli.³

Packaging

MacConkey Agar w/o Crystal Violet	Code No.	7236A	500 g
		7236B	2 kg
		7236C	10 kg

References

1. **MacConkey, A.** 1905. Lactose-fermenting bacteria in feces. J. Hyg. 5:333-379.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Mazura-Reetz, G. T. Neblett, and J. M. Galperin.** 1979. MacConkey Agar: CO₂ vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MacCONKEY AGAR w/o CRYSTAL VIOLET & SALT (7378)

Intended Use

MacConkey Agar w/o Crystal Violet & Salt is used for the isolation and differentiation of gram-negative enteric bacilli from specimens containing swarming strains of *Proteus* spp.

Product Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.¹ The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula modifications improved differential reactions between enteric pathogens and coliforms.

MacConkey Agar w/o Crystal Violet & Salt is a differential medium that restricts swarming of *Proteus* spp., aiding in the detection and isolation of enteric microorganisms. Sodium Chloride is deleted from the medium to provide an electrolyte deficient medium preventing *Proteus* spp. from spreading. In addition, this medium does not contain crystal violet allowing *Staphylococcus*, *Enterococcus*, and *Mycobacterium* spp. to grow.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar w/o Crystal Violet & Salt. Lactose is the fermentable carbohydrate. During Lactose fermentation, a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation develops. Bile Salts Mixture is a selective agent. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	18.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Lactose	10 g
Bile Salts Mixture	5 g
Neutral Red.....	0.04 g
Agar	12 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 47 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and trace to light pink-beige.

Prepared Appearance: Prepared medium is clear to slightly hazy, and light to medium red-brown to red-orange.

Expected Cultural Response: Cultural response on MacConkey Agar w/o Crystal Violet & Salt at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	growth	pink colonies
<i>Escherichia coli</i> ATCC® 25922	growth	bright pink colonies
<i>Proteus mirabilis</i> ATCC® 12453	growth	translucent colonies, colorless, no swarming
<i>Salmonella typhimurium</i> ATCC® 14028	growth	colorless colonies
<i>Shigella sonnei</i> ATCC® 25931	growth	colorless colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references using MacConkey Agar w/o Crystal Violet & Salt for the isolation and identification of enteric organisms.²

Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or without a zone of precipitated bile. Non-lactose fermenting organisms grow as colorless or clear colonies. Swarming by *Proteus* spp. is reduced.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Although MacConkey Agar w/o Crystal Violet & Salt is a selective medium, it is less inhibitory than MacConkey Agar, allowing gram-positive organisms to grow. Biochemical and serological testing using pure cultures are recommended for complete identification.
3. Incubation of MacConkey Agar w/o Crystal Violet & Salt under increased CO₂ has been reported to reduce growth and recovery of certain gram-negative bacilli.³

Packaging

MacConkey Agar w/o Crystal Violet & Salt	Code No.	7378A	500 g
		7378B	2 kg
		7378C	10 kg

References

1. **MacConkey, A.** 1905. Lactose-fermenting bacteria in feces. J. Hyg. 5:333-379.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Mazura-Reetz, G. T. Neblett, and J. M. Galperin.** 1979. MacConkey Agar: CO₂ vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MacCONKEY AGAR w/ SORBITOL (7320)

Intended Use

MacConkey Agar w/ Sorbitol is used for the isolation of pathogenic *Escherichia coli*.

Product Summary and Explanation

MacConkey Agar w/ Sorbitol is based on the formula by Rappaport and Henig.¹ Originally developed for isolating enteropathogenic (EPEC) *serotypes*, O11 and O55, this medium is recommended for the isolation and differentiation of enterohemorrhagic *E. coli* O157:H7. This organism causes hemorrhagic colitis, which results in bloody diarrhea and can lead to kidney failure and death.² Serotype O157 has been implicated in serious foodborne diseases.

MacConkey Agar w/ Sorbitol contains sorbitol instead of lactose for differentiating enteropathogenic *E. coli* serotypes; these strains are typically sorbitol negative. MacConkey Agar w/ Sorbitol is recommended for clinical and food testing.²⁻⁴

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar w/ Sorbitol. Sorbitol is the fermentable carbohydrate; typically enteropathogenic strains produce colorless colonies. Bile Salts Mixture and Crystal Violet are the selective agents, inhibiting Gram-positive cocci. Sodium Chloride maintains the osmotic environment, and Neutral Red is the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Sorbitol.....	10 g
Bile Salts Mixture	1.5 g
Sodium Chloride	5 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.001 g
Agar	13.5 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 50 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and dispense into sterile Petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pink-beige.

Prepared Appearance: Prepared medium is red-purple, slightly opalescent.

Expected Cultural Response: Cultural response on MacConkey Agar w/ Sorbitol at 37°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	good growth, pink colonies are sorbitol positive
<i>Escherichia coli</i> ATCC® 35150	good growth, colorless colonies are sorbitol negative
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to the appropriate references for specific procedures using MacConkey Agar w/ Sorbitol.²⁻⁴

Results

E. coli O157:H7, and other organisms that do not ferment sorbitol, are colorless on MacConkey Agar w/ Sorbitol. Sorbitol-fermenting organisms produce pink colonies. Confirmatory biochemical and serological testing should be performed on suspected colonies.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Colonies that are sorbitol positive can revert, and can be mistaken for sorbitol negative.⁵
3. *E. coli* O157:H7 can ferment sorbitol after prolonged incubation.⁵

Packaging

MacConkey Agar w/ Sorbitol	Code No.	7320A	500 g
		7320B	2 kg
		7320C	10 kg

References

1. **Rappaport, F., and E. Henig.** 1952. Media for the isolation and differentiation of pathogenic *Escherichia coli* (serotypes 0111 and 055). J. Clin. Pathol. **5**:361-362.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Adams, S.** 1991. Screening for verotoxin-producing *Escherichia coli*. Clin. Lab. Sci. **4**:19-20.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MacCONKEY AGAR, CS (7391)

Intended Use

MacConkey Agar, CS is used for the isolation and differentiation of gram-negative enteric bacilli from specimens containing swarming strains of *Proteus* spp.

Product Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.¹ The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula modifications improved differential reactions between enteric pathogens and coliforms.

MacConkey Agar, CS ("Controlled Swarming") contains carefully selected raw materials to reduce swarming of *Proteus* spp., which could cause difficulty in isolating and enumerating other gram-negative bacilli.

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar, CS. Lactose is the fermentable carbohydrate. During Lactose fermentation a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation. Bile Salts and Crystal Violet are the selective agents, inhibiting gram-positive cocci and allowing gram-negative organisms to grow. Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Lactose	10 g
Bile Salts	1.5 g
Sodium Chloride	5 g
Neutral Red	0.03 g
Crystal Violet.....	0.001 g
Agar	13.5 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 50 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light pink-beige.

Prepared Appearance: Prepared medium is dark pink-purple and clear to slightly hazy.

Expected Cultural Response: Cultural response on MacConkey Agar, CS at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	pink colonies
<i>Proteus mirabilis</i> ATCC® 12453	growth	colorless colonies, swarming inhibited
<i>Salmonella typhimurium</i> ATCC® 14028	growth	colorless colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references using MacConkey Agar, CS for the isolation and identification of enteric organisms.²

Results

Lactose-fermenting organisms grow as pink to brick red colonies with or without a zone of precipitated bile. Non-lactose fermenting organisms grow as colorless or clear colonies. Swarming by *Proteus* spp. is reduced on MacConkey Agar, CS.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Although MacConkey Agar, CS is a selective medium primarily for gram-negative enteric bacilli, biochemical and serological testing using pure cultures are recommended for complete identification.
3. Incubation of MacConkey Agar, CS under increased CO₂ has been reported to reduce the growth and recovery of certain strains of Gram-negative bacilli.³

Packaging

MacConkey Agar, CS	Code No.	7391A	500 g
		7391B	2 kg
		7391C	10 kg

References

1. **MacConkey, A.** 1905. Lactose-fermenting bacteria in feces. J. Hyg. 5:333-379.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Mazura-Reetz, G. T. Neblett, and J. M. Galperin.** 1979. MacConkey Agar: CO₂ vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MacCONKEY AGAR, MODIFIED (7440)

Intended Use

MacConkey Agar, Modified is used for determining the fecal coliform count in seawater and shellfish.

Product Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.¹ The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. This formula was modified to improve growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula modifications improved differential reactions between enteric pathogens and coliforms.

The U.S. Department of Health, Education, and Welfare² and the APHA³ recommend MacConkey Agar, Modified for the determination of the fecal coliform content in seawater and shellfish. This formulation differs from MacConkey Agar by omitting sodium chloride and lowering the content of bile salts.

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar, Modified. Lactose is the fermentable carbohydrate. During Lactose fermentation a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation. Bile Salts and Crystal Violet are the selective agents, inhibiting Gram-positive cocci and allowing Gram-negative organisms to grow. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Lactose	10 g
Bile Salts	0.75 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.001 g
Agar	13.5 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 44.25 g of the medium in 500 mL of purified water.
2. Heat with frequent agitation to boiling and remove from heat. Boil again.
3. DO NOT AUTOCLAVE.
4. Maintain the medium at 55 - 60°C for no longer than 4 hours.
5. Mix with equal volume of seawater or shellfish sample. For seawater samples, also add 0.12 g of glycine.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light pink-beige.

Prepared Appearance: Prepared medium is medium to dark pinkish-purple and trace to slightly hazy.

Expected Cultural Response: Cultural response on unsupplemented MacConkey Agar, Modified at 45.5°C after 18 - 30 hours incubation.

Microorganism	Response	Reactions
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	pink colonies
<i>Escherichia coli</i> ATCC® 11775	growth	pink colonies with bile ppt
<i>Escherichia coli</i> ATCC® 25922	growth	pink colonies with bile ppt
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Seawater Samples

1. Mix 60 mL of seawater and 0.12 mL glycine together with 60 mL of molten medium.
2. Dispense into six petri dishes.
3. Incubate petri dishes for 18 - 30 hours at 45.5°C ± 0.5.

Shellfish Samples

1. Six grams of shellfish homogenate are brought to a 60 mL volume with phosphate buffered saline.
2. Mix shellfish homogenate mixture with 60 mL of the molten medium.
3. Pour blended mixture into 6 petri dishes.
4. Incubate petri dishes for 18 - 30 hours at 45.5°C ± 0.5.

For a complete discussion on test procedures refer to appropriate references.^{2,3}

Results

Count and record only red colonies with red or pink halos as fecal coliforms.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

MacConkey Agar, Modified	Code No.	7440A	500 g
		7440B	2 kg
		7440C	10 kg

References

1. **MacConkey, A.** 1905. Lactose-fermenting bacteria in feces. J. Hyg. 5:333-379.
2. **Interim Guides for the Depuration of the Northern Quahuag, *Mercenaria mercenaria*.** 1968. Supplement I-Part IV of the National Shellfish Manual of Operations. H.E.W. Northeast Marine Health Sciences Laboratory.
3. **American Public Health Association.** 1985. Laboratory procedures for the examination of seawater and shellfish, 5th ed. APHA, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MacCONKEY BROTH (7185)

Intended Use

MacConkey Broth is used for the detection of coliform bacteria in milk and water.

Product Summary and Explanation

MacConkey Broth is a modification of the original bile salt broth recommended by MacConkey, containing 0.5% sodium taurocholate and litmus as an indicator.¹ MacConkey suggested further variations of this formula using neutral red indicator instead of litmus.^{2,3} Childs and Allen demonstrated the inhibitory effect of neutral red and substituted the less inhibitory Bromcresol purple.⁴

Principles of the Procedure

Enzymatic Digest of Gelatin provides the nitrogen and vitamin sources in MacConkey Broth. Lactose is the carbohydrate for Gram-negative lactose-fermenting bacilli. Bile Salts inhibit the growth of gram-positive organisms. Bromcresol Purple is the indicator.

Formula / Liter

Enzymatic Digest of Gelatin 20 g
Lactose 10 g
Bile Salts 5 g
Bromcresol Purple 0.01 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 35 g of the medium in one liter of purified water.
2. Dispense into tubes containing Durham tubes.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light beige.

Prepared Appearance: Prepared medium is dark purple to red-purple, and clear to slight hazy.

Expected Cultural Response: Cultural response in MacConkey Broth at 35°C after 24 hours incubation.

Microorganism	Response	Reactions	
		Acid	Gas
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	positive	positive
<i>Escherichia coli</i> ATCC® 11775	growth	positive	positive
<i>Escherichia coli</i> ATCC® 25922	growth	positive	positive
<i>Salmonella typhimurium</i> ATCC® 14028	growth	negative	negative
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to the appropriate references using MacConkey Broth.

Results

Lactose-fermenting organisms grow well in MacConkey Broth and produce acid, causing the medium to turn yellow. Gas is also produced. Non-fermenting organisms produce good growth, but will not produce acid or gas.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

MacConkey Broth	Code No.	7185A	500 g
		7185B	2 kg
		7185C	10 kg

References

1. **MacConkey, A.** 1901. Centr. Bakt. **29**:740.
2. **MacConkey, A.** 1905. Lactose-fermenting bacteria in faeces. J. Hyg. **5**:333-379.
3. **MacConkey, A.** 1908. Bile salt media and their advantage in some bacteriological examinations. J. Hyg. **8**:322-
Streptococcus faecalis. J. Hyg. Camb. **51**:468-477.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MALONATE BROTH (7516)

Intended Use

Malonate Broth is used for the differentiation of *Enterobacter* spp. and *Escherichia* spp. on the basis of malonate utilization.

Product Summary and Explanation

Malonate Broth is prepared according to the formula described by Leifson.¹ Malonate Broth is a liquid medium containing ammonium sulfate as the only source of nitrogen, and malonate as the only source of carbon. During Leifson's experiments the *Enterobacter* group utilized malonate, producing an alkaline reaction and changing the color of the medium from green to blue. The *Escherichia* group did not utilize malonate (medium remained green) and failed to grow.

Malonate Broth is further described for differentiating *Enterobacteriaceae* in food and dairy products.²⁻⁴ In certain cases the medium referenced is the Edwards and Ewing formulation that contains yeast extract and dextrose.⁵ Adding yeast extract and dextrose permits growth of organisms that would otherwise fail in Malonate Broth.

Principles of the Procedure

Ammonium Sulfate is the sole source of nitrogen in Malonate Broth. Sodium Malonate is the carbon source. 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 / Liter

Ammonium Sulfate	2 g
Dipotassium Phosphate	0.6 g
Monopotassium Phosphate	0.4 g
Sodium Chloride	2 g
Sodium Malonate	3 g
Bromthymol Blue.....	0.025 g

Final pH: 6.7 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. HARMFUL. Irritating to eyes, respiratory system and skin.

Directions

1. Dissolve 8 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and yellow to green-beige.

Prepared Appearance: Prepared medium is clear and green with red highlights.

Expected Cultural Response: Cultural response in Malonate Broth at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Enterobacter aerogenes</i> ATCC® 13048	fair to good growth	blue
<i>Escherichia coli</i> ATCC® 25922	fair to good growth	green
<i>Klebsiella pneumoniae</i> ATCC® 13883	fair to good growth	blue
<i>Salmonella arizonae</i> ATCC® 13314	fair to good growth	blue
<i>Salmonella typhimurium</i> ATCC® 14028	fair to good growth	green

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate tubes with a loopful of test organism. Incubate at 35 ± 2°C for 18 – 48 hours. Examine tubes for a change in the color of the medium from green to blue.

Results

Malonate utilization is indicated by a change in the color of the medium from green to blue,

Positive: Blue

Negative: Green

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

A slight bluing (blue-green) of the medium may occur after prolonged incubation.⁶ In such cases, care should be taken in interpreting results.

Packaging

Malonate Broth	Code No.	7516A	500 g
		7516B	2 kg
		7516C	10 kg

References

1. **Leifson, E.** 1933. The fermentation of sodium malonate as a means of differentiating *Aerobacter* and *Escherichia*. J. Bacteriol. **26**:329.
2. **Bacteriological analytical manual.** 1995. 8th ed. AOAC International, Arlington, VA.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Edwards, P. R., and W. H. Ewing.** 1962. *Enterobacteriaceae*. U. S. Public Health Service Bulletin No. **734**:19.
6. **Oberhofer, T. R.** 1985. Manual of nonfermenting Gram-negative bacteria. Churchill Livingstone, New York, NY.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MALT AGAR (7456)

Intended Use

Malt Agar is used for the cultivation of fungi.

Product Summary and Explanation

Malt media for yeasts and molds have been used for many years. In 1919, Reddish prepared a satisfactory substitute for beer wort from malt extract.¹ Thom and Church used Reddish's medium for their studies of aspergilli.² Malt Agar was employed by Fullmer and Grimes during studies of yeasts on synthetic media.³ Malt Agar is specified in standard methods for the examination of yeasts and molds.^{4,5}

Principles of the Procedure

Malt Extract provides carbon, protein, and nitrogen sources required for organism growth. Agar is a solidifying agent. The acidic pH of Malt Agar allows for optimal growth of molds and yeasts while restricting bacterial growth.

Formula / Liter

Malt Extract 30 g

Agar 15 g

Final pH: 5.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 45 g of the medium in one liter of purified water.
2. Heat with frequent agitation to boiling to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light tan.

Prepared Appearance: Prepared medium is clear to trace hazy and yellow-tan in color.

Expected Cultural Response: Cultural response on Malt Agar at 25°C after 2 - 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Do not heat the medium after addition of acid. The agar will hydrolyze, reducing the agar's solidifying properties.

Packaging

Malt Agar	Code No.	7456A	500 g
		7456B	2 kg
		7456C	10 kg

References

1. **Abs.** 1919. Bact. 3:6.
2. **Thom, C., and M. B. Church.** 1926. The Aspergilli. Williams and Wilkins Co., Baltimore, MD.
3. **Fullmer, E. I., and M. J. Grimes.** 1923. The growth of yeasts on synthetic agar media. Bacteriol. 8:585-588.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. America Public Health Association, Washington, D.C.
5. **Association of Official Agricultural Chemists.** 1995. Official methods of analysis, 16th ed. AOAC, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MALT EXTRACT (7341)

Intended Use

Malt Extract is a dehydrated extract of malt for use in preparing microbiological culture media.

Product Summary and Explanation

Malt Extract is a clarified water soluble extract of malted barley. Malt Extract is a useful ingredient of culture media designed for the propagation of yeasts and molds. This ingredient is suitable for yeasts and molds because it contains a high concentration of carbohydrates, particularly maltose. The approximate percentage of reducing sugars in Malt Extract is 60 – 63%. Malt Extract is generally employed in culture media at concentrations between 10 to 100 grams per liter.

Malt Agar, a medium recommended for the detection and isolation of yeast and molds from dairy products, food, and as a stock culture, contains Malt Extract. Wort Agar, used for the cultivation and enumeration of yeasts, has Malt Extract as one of the main ingredients in the formula. Several media containing Malt Extract are specified in standard methods.¹⁻³

Principles of the Procedure

Malt Extract provides carbon, protein, and nutrients for the isolation and cultivation of yeasts and molds in microbiological culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and light beige to tan.

Prepared Appearance (2% wt/vol): Prepared medium is clear, dark amber with no or a light precipitate.

pH (2% Solution at 25°C): 4.5 - 5.7

Expected Cultural Response: Cultural response on Malt Agar after incubation at 25 - 35°C for up to 7 days.

Microorganism	Response
<i>Aspergillus niger</i> ATCC 16404	good to excellent growth with sporulation
<i>Candida albicans</i> ATCC 10231	good to excellent growth

Test Procedure

Refer to appropriate references for specific procedures using Malt Extract.

Results

Refer to appropriate references for test results.

Storage

Store sealed container of Malt Extract at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Malt Extract should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Malt Extract in its intact container when stored as directed.

Packaging**Malt Extract**

Code No.	7341A	500 g
	7341B	2 kg
	7341C	10 kg

References

1. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.
2. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MALT EXTRACT AGAR (7303)

Intended Use

Malt Extract Agar is used for the cultivation of fungi.

Product Summary and Explanation

The use of malt and malt extract for the propagation of yeasts and molds is quite common. In 1919, Reddish prepared a satisfactory substitute for beer wort from malt extract.¹ Thom and Church, following the formula of Reddish, used malt extract as a base to prepare the complete medium.²

Principles of the Procedure

Maltose is the energy source in Malt Extract Agar. Dextrin is a polysaccharide derived from high quality starch. Glycerol is included as an energy source. Enzymatic Digest of Gelatin provides nitrogen. Agar is a solidifying agent. The acidic pH of Malt Extract Agar allows for optimal growth of molds and yeasts while restricting bacterial growth.

Formula / Liter

Maltose	12.75 g
Dextrin.....	2.75 g
Glycerol.....	2.35 g
Enzymatic Digest of Gelatin.....	0.78 g
Agar	15 g

Final pH: 4.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 33.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 118 - 121°C for 15 minutes. DO NOT OVERHEAT.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is moderately hazy and grey-white.

Expected Cultural Response: Cultural response on Malt Extract Agar at 25 - 30°C after 2 - 7 days of incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Malt Extract Agar	Code No.	7303A	500 g
		7303B	2 kg
		7303C	10 kg

References

1. **ABS.** 1919. Bact. 3:6.
2. **Thom, C., and M. B. Church.** 1926. The Aspergilli. Williams and Wilkins Co., Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MANNITOL SALT AGAR (7143)

Intended Use

Mannitol Salt Agar is used for the isolation of staphylococci.

Product Summary and Explanation

Chapman formulated Mannitol Salt Agar to isolate staphylococci by inhibiting growth of most other bacteria with a high salt concentration.¹ Chapman added 7.5% Sodium Chloride to Phenol Red Mannitol Agar, and noted pathogenic strains of staphylococci (coagulase-positive staphylococci) grew luxuriantly and produced yellow colonies with yellow zones. Nonpathogenic staphylococci produced small red colonies with no color change to the surrounding medium.

Mannitol Salt Agar is highly selective, and specimens from heavily contaminated sources may be streaked onto this medium without danger of overgrowth.² Mannitol Salt Agar is recommended for isolating pathogenic staphylococci from clinical specimens, cosmetics, and microbial limit tests.²⁻⁴

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide the nitrogen, vitamins, and carbon in Mannitol Salt Agar. D-Mannitol is the carbohydrate source. In high concentrations, Sodium Chloride inhibits most bacteria other than staphylococci. Phenol Red is the pH indicator. Agar is the solidifying agent.

Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products, turning the Phenol Red pH indicator from red to yellow. Typical pathogenic staphylococci ferment mannitol and form yellow colonies with yellow zones. Typical non-pathogenic staphylococci do not ferment mannitol and form red colonies.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Beef Extract	1 g
D-Mannitol.....	10 g
Sodium Chloride	75 g
Phenol Red	0.025 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 111 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to trace hazy and yellow or peach to light pink.

Expected Cultural Response: Cultural response on Mannitol Salt Agar at 35°C after 24 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Proteus mirabilis</i> ATCC® 12453	partial inhibition	--
<i>Staphylococcus aureus</i> ATCC® 25923	growth	yellow colonies with yellow zones
<i>Staphylococcus epidermidis</i> ATCC® 12228	growth	pink colonies with no zone of color change

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate specimen on medium as a primary isolation or inoculate isolated colonies onto medium for differentiation.

Results

Staphylococci will grow on this medium, while the growth of most other bacteria will be inhibited. Coagulase-positive staphylococci will produce luxuriant growth of yellow colonies with yellow zones. Coagulase-negative staphylococci will produce small pink to red colonies with no color change to medium.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Mannitol Salt Agar	Code No.	7143A	500 g
		7143B	2 kg
		7143C	10 kg

References

1. **Chapman, G. H.** The significance of sodium chloride in studies of staphylococci. *J. bacteriol.* **50**:201.
2. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1995. Microbiology methods for cosmetics, p. 23.01-23.12. In *Bacteriological analytical manual*, 8th ed. AOAC International, Gaithersburg, MD.
4. **United States Pharmacopeial Convention.** 1995. *The United States pharmacopeia*, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MCP AGAR (7477)

Intended Use

MCP Agar is used with additives for the enumeration of *Clostridium perfringens* in environmental samples.

Product Summary and Explanation

MCP Agar was described by Bisson & Capbelli¹ and modified by Armon and Payment.² This medium is used to enumerate *Clostridium perfringens* spores from surface and drinking water. *C. perfringens* is present in large numbers in human and animal wastes. *C. perfringens* spores are resistant to wastewater treatment practices and environmental stresses. Growth of *C. perfringens* spores is an excellent indicator of present and past fecal contamination.

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, amino acids, and carbon required for organism growth in MCP Agar. Yeast Extract supplies essential vitamins. Sucrose is the fermentable carbohydrate. L-Cysteine•HCl is a reducing agent and Magnesium Sulfate (MgSO₄) provides trace ions. Bromcresol Purple is the pH indicator and Agar is the solidifying agent. MCP is supplemented with D-Cycloserine, Polymyxin B Sulfate, and FeCl₃•6H₂O as selective agents. Phenolphthalein Diphosphate is the substrate used for detection of an acid phosphatase enzyme elaborated by *C. perfringens*. In the presence of this enzyme and ammonium hydroxide fumes, the diphosphate bond is cleaved. This reaction is visible by the absorption of Indoxyl-β-D-Glucoside, producing red to dark pink colonies.

Formula / Liter

Enzymatic Digest of Casein	30 g
Yeast Extract.....	20 g
Sucrose	5 g
L-Cysteine•HCl	1 g
MgSO ₄ •7H ₂ O	0.1 g
Bromcresol Purple	0.04 g
Agar	15 g

Final pH: 7.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

D-Cycloserine, 0.4 g
Polymyxin B Sulfate, 0.025 g
FeCl ₃ •6H ₂ O, 2 mL, 4.5%
Phenolphthalein Diphosphate, 20 mL 0.5%
Indoxyl-β-D-Glucoside, 80 mL, 0.075%

Precaution

1. For Laboratory Use.

Directions

1. Suspend 71.1 g of the medium in 900 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 50°C.
4. Aseptically add 0.4 g of D-cycloserine, 0.025 g of polymyxin B sulfate, 2 mL of a filter sterilized 4.5% FeCl₃•6H₂O solution, 20 mL of a filter sterilized 0.5% phenolphthalein diphosphate solution, and 80 mL of a filter sterilized 0.075% indoxyl-β-D-glucoside solution.
5. Mix thoroughly and dispense into appropriate petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and purple to dark purple.

Expected Cultural Response: Cultural response on MCP Agar with supplements after 18 – 24 hours incubation at 35°C under anaerobic conditions.

Microorganism	Response	Reactions
<i>Clostridium perfringens</i> ATCC® 13124	growth	yellow colonies
<i>Clostridium sporogenes</i> ATCC® 11437	growth	clear colonies
<i>Clostridium novyi</i> ATCC® 7659	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Pass the water sample through a membrane filter.
2. Place the filter on MCP agar and incubate anaerobically for 18 – 24 hours at 44.5°C.
3. The presence of yellow colonies indicates sucrose fermentation and indicative of *C. perfringens*.
4. Expose the filter to ammonium hydroxide fumes for 20 seconds by removing the plate lid and inverting the plate surface over an open container of concentrated ammonia hydroxide. Use proper technique and perform this portion of the test in a hood that will ventilate to the outside.
5. Count red to dark pink colonies.

Results

Red or dark pink colonies (acid phosphatase cleavage of phenolphthalein diphosphate) are counted as presumptive of *C. perfringens*.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. MCP is a presumptive test for the presence of *C. perfringens*. Biochemical tests are required for complete identification of *C. perfringens*.

Packaging

MCP Agar	Code No.	7477A	500 g
		7477B	2 kg
		7477C	10 kg

References

1. **Appl. and Environ. Microbiol.** 1979. **37**:5-56.
2. **Canadian J. Microbiol.** 1988. **31**:78-79.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MIDDLEBROOK 7H10 AGAR (7246)

Intended Use

Middlebrook 7H10 Agar is used with glycerol and OADC Enrichment for the cultivation of *Mycobacterium* spp.

Product Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.¹ Non-tuberculous mycobacteria infections have also increased since 1985.² There are two types of solid culture media for the primary isolation of mycobacteria, coagulated egg as a base (Lowenstein formulations) and an agar base (Middlebrook formulations). The use of agar-based media for primary isolation of mycobacteria have the following significant advantages:

1. Agar-based media do not usually liquefy in the presence of contaminating proteolytic organisms.²
2. Agar-based media are recommended for specimens from nonsterile sites, because colonies of mycobacteria can be viewed in a clear medium after 10 – 12 days incubation using a stereo microscope even if contaminating organisms are present.³
3. Middlebrook formulations retain exact concentrations of added drugs because the medium is solidified with agar rather than by inspissation of the egg. There is less drug inactivation when egg ingredients are absent.

Middlebrook 7H10 Agar is prepared according to Middlebrook, Cohn, Dye, Russell, and Levy.⁴ This medium contains a low concentration of malachite green, which may be preferable for primary isolation.

Principles of the Procedure

Ammonium Sulfate, Sodium Citrate, Pyridoxine, Monosodium Glutamate, and Biotin supply growth factors. Magnesium Sulfate, Ferric Ammonium Citrate, Zinc Sulfate, Calcium Chloride, and Copper Sulfate are sources of trace ions. Disodium Phosphate and Monopotassium Phosphate help maintain the pH of the medium. Malachite Green inhibits contaminating organisms. Agar is a solidifying agent. Glycerol enhances growth of *Mycobacterium avium* and other *Mycobacterium* spp.³ OADC Enrichment contains Dextrose and Oleic Acid as carbon sources.

Formula / Liter

Disodium Phosphate.....	1.5 g
Monopotassium Phosphate	1.5 g
Ammonium Sulfate	0.5 g
Monosodium Glutamate.....	0.5 g
Sodium Citrate	0.4 g
Ferric Ammonium Citrate.....	0.04 g
Magnesium Sulfate	0.05 g
Copper Sulfate	0.001g
Pyridoxine	0.001 g
Zinc Sulfate	0.001 g
Biotin	0.0005 g
Calcium Chloride.....	0.0005 g
Malachite Green.....	0.00025 g
Agar	13.5 g

Final pH: 6.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Glycerol, 5 mL
OADC Enrichment, 100 mL

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 18 g of the medium in 900 mL of purified water containing 5 mL of glycerol.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Cool to 45 - 50°C and aseptically add 100 mL of OADC Enrichment.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and blue-beige.

Prepared Appearance: Prepared medium is slightly opalescent and grey-white.

Expected Cultural Response: Cultural response on Middlebrook 7H10 Agar at 35°C after 2 – 3 weeks incubation.

Microorganism	Response
<i>Mycobacterium tuberculosis</i> ATCC® 25177	growth
<i>Mycobacterium kansasii</i> ATCC® 12478	growth
<i>Mycobacterium scrofulaceum</i> ATCC® 19981	growth
<i>Mycobacterium intracellulare</i> ATCC® 13950	growth
<i>Mycobacterium fortuitum</i> ATCC® 6841	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate the specimen onto the medium. Incubate tubes for up to eight weeks. Examine tubes for growth. Refer to specific procedures for a complete discussion on the isolation and identification of *Mycobacterium* spp.

Results

Observe for colonies that may or may not be pigmented. Colony morphology is dependent on the species isolated.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Mycobacterium* spp.
2. Negative culture results do not rule out an active mycobacterial infection.

Packaging

Middlebrook 7H10 Agar	Code No.	7246A	500 g
		7246B	2 kg
		7246C	10 kg

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria: molecular genetic insights. *Clinical Microbiology Reviews*. **8**:496-514.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Isenberg, H. D. (ed.).** 1992. *Clinical microbiology procedures handbook*, vol. 1 American Society for Microbiology, Washington, D.C.
4. **Middlebrook, G., M. L. Cohn, W. B. Dye, W. B. Russell, Jr., and D. Levy.** 1960. Microbiological procedures of value in tuberculosis. *Acta. Tubercul. Scand.* **38**:66.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MIDDLEBROOK 7H11 AGAR (7244)

Intended Use

Middlebrook 7H11 Agar is used with glycerol and OADC Enrichment for the cultivation of *Mycobacterium* spp.

Product Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.¹ Non-tuberculous mycobacteria infections have also increased since 1985.² There are two types of solid culture media for the primary isolation of mycobacteria, coagulated egg as a base (Lowenstein formulations) and an agar base (Middlebrook formulations). The use of agar-based media for primary isolation of mycobacteria have the following significant advantages:

1. Agar-based media do not usually liquefy in the presence of contaminating proteolytic organisms.²
2. Agar-based media are recommended for specimens from nonsterile sites, because colonies of mycobacteria can be viewed in a clear medium after 10 – 12 days incubation using a stereo microscope even if contaminating organisms are present.³
3. Agar-based media retain exact concentrations of added drugs because the medium is solidified with agar rather than by inspissation of the egg. There is less drug inactivation when egg ingredients are absent.

Middlebrook 7H11 Agar is a modification of Middlebrook 7H10 Agar Special as recommended by Cohn, Waggoner, and McClately.⁴ Cohn et al. added an enzymatic digest of casein and found organism growth was stimulated for fastidious strains of *Mycobacterium tuberculosis* and provided improved susceptibility testing.⁴

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, vitamins, and amino acids in Middlebrook 7H11 Agar. Ammonium Sulfate, Sodium Citrate, Pyridoxine, Monosodium Glutamate, and Biotin supply growth factors. Magnesium Sulfate, Ferric Ammonium Citrate, Zinc Sulfate, and Copper Sulfate are sources of trace ions required for growth of *Mycobacteria* spp. Disodium Phosphate and Monopotassium Phosphate help maintain the pH of the medium. Malachite Green inhibits contaminating organisms. Agar is a solidifying agent. Glycerol enhances the growth of *Mycobacterium avium* and other *Mycobacterium* spp.³ OADC Enrichment contains Dextrose and Oleic Acid as carbon sources.

Formula / Liter

Enzymatic Digest of Casein	1 g
Disodium Phosphate.....	1.5 g
Monopotassium Phosphate	1.5 g
Ammonium Sulfate	0.5 g
Monosodium Glutamate.....	0.5 g
Sodium Citrate	0.4 g
Ferric Ammonium Citrate.....	0.04 g
Magnesium Sulfate	0.05 g
Copper Sulfate	0.001 g
Pyridoxine	0.001 g
Zinc Sulfate	0.001 g
Biotin	0.0005 g
Malachite Green.....	0.00025 g
Agar	13.5 g

Final pH: 6.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Glycerol, 5 mL
OADC Enrichment, 100 mL

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 19 g of the medium in 900 mL of purified water containing 5 mL of glycerol.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Cool to 45 - 50°C and aseptically add 100 mL of OADC Enrichment.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is slightly opalescent and light greyish-white.

Expected Cultural Response: Cultural response on Middlebrook 7H11 Agar at 35°C after 2 – 3 weeks incubation.

Microorganism	Response
<i>Mycobacterium fortuitum</i> Group IV ATCC® 6841	growth
<i>Mycobacterium intracellulare</i> Group III ATCC® 13950	growth
<i>Mycobacterium kansasii</i> Group I ATCC® 12478	growth
<i>Mycobacterium scrofulaceum</i> Group II ATCC® 19981	growth
<i>Mycobacterium tuberculosis</i> H37Ra ATCC® 25177	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate specimen onto the medium. Incubate tubes for up to eight weeks. Examine tubes for growth at regular intervals. Refer to specific procedures for a complete discussion on the isolation and identification of *Mycobacterium* spp.

Results

Observe colonies that may or may not be pigmented. Colony morphology is dependent on the species isolated.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Mycobacterium* spp.
2. Negative culture results do not rule out an active mycobacterial infection.

Packaging

Middlebrook 7H11 Agar	Code No.	7244A	500 g
		7244B	2 kg
		7244C	10 kg

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria: molecular genetic insights. *Clinical Microbiology Reviews*. **8**:496-514.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Isenberg, H. D. (ed.).** 1992. *Clinical microbiology procedures handbook*, vol. 1 American Society for Microbiology, Washington, D.C.
4. **Cohn, M. L., R. F. Waggoner, and J. K. McClatchy.** 1968. The 7H11 Medium for the cultivation of mycobacteria. *Am. Rev. Resp. Dis.* **98**:295.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MIO MEDIUM (7389)

Intended Use

MIO Medium is used for the differentiation of microorganisms on the basis of motility, ornithine decarboxylase activity, and indole production.

Product Summary and Explanation

Tests for indole production, motility, and ornithine decarboxylase activity play important roles in the identification of *Enterobacteriaceae*. Ederer and Clark¹ and Oberhofer and Hajkowski² developed MIO Medium, combining all three differentiating reactions in one medium. Ederer and Clark stressed the advantages of MIO Medium in their extensive study comparing cultural reactions of *Enterobacteriaceae* on MIO Medium with reactions on classic media.¹

Principles of the Procedure

The nitrogen, carbon, and amino acids sources are provided by Enzymatic Digest of Gelatin and Enzymatic Digest of Casein. Yeast Extract provides vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon. Dextrose is an energy source. The small concentration of agar is added to demonstrate motility. All *Enterobacteriaceae* ferment dextrose. Fermentation lowers the pH, causing MIO Medium to change from purple to yellow. If the organism possesses ornithine decarboxylase, L-Ornithine is decarboxylated to putrescine, causing the pH to increase and changing the color of the medium from yellow to purple. The pH indicator, Bromcresol Purple, facilitates detection of decarboxylase activity.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Enzymatic Digest of Casein	10 g
Yeast Extract.....	3 g
Dextrose.....	1 g
Bromcresol Purple	0.02 g
L-Ornithine	5 g
Agar	2 g

Final pH: 6.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 31 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and very pale to light green-beige.

Prepared Appearance: Prepared medium is clear to trace hazy and purple.

Expected Cultural Response: Cultural response in MIO Medium at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions		
		Motility	Indole	Ornithine
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	positive	negative	positive
<i>Escherichia coli</i> ATCC® 25922	growth	positive	positive	positive
<i>Klebsiella pneumoniae</i> ATCC® 13883	growth	negative	negative	negative
<i>Proteus mirabilis</i> ATCC® 12453	growth	variable	negative	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Using a wire, inoculate medium with stab motion to the bottom of the tube with isolated colonies.
2. Incubate with loose caps at $35 \pm 2^{\circ}\text{C}$ for 18 - 48 hours.
3. Examine tubes at 18 - 24 hours for growth, color change, and motility. Re-examine tubes at 40 - 48 hours.
4. Add 3 - 4 drops of Kovac's Reagent to each tube. Record as indole positive if a pink or red color appear, or as indole negative if there is no color change. Add Kovac's Reagent after determining motility and ornithine decarboxylase reactions.

Results

Motility is indicated by turbidity of the medium or growth extending from inoculating stab line. A purple color throughout the medium indicates a positive ornithine reaction. (The color may vary in intensity.) If the organism is ornithine negative, the medium is yellow. Indole is detected by adding Kovac's Reagent to the surface of the medium. A pink or red color indicates an indole-positive culture. Indole is produced from the tryptophane present in the medium.

Refer to appropriate references for complete identification of *Enterobacteriaceae*.³

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

MIO Medium	Code No.	7389A	500 g
		7389B	2 kg
		7389C	10 kg

References

1. **Ederer, G. M. and M. Clark.** 1970. Motility-Indole-Ornithine medium. Appl Microbiol. **2**:849.
2. **Oberhofer, T. R., and R. Hajkowski.** 1970. Evaluation of non-lactose-fermenting members of the *Klebsiella-Enterobacter-Serratia* Division. I. Biochemical characteristics. Am. J. Clin. Pathol. **54**:720.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover. (eds.).** 1995. Manual of clinical microbiology. 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MITIS SALIVARIUS AGAR (7277)

Intended Use

Mitis Salivarius Agar is used for the isolation of *Streptococcus mitis*, *Streptococcus salivarius*, and enterococci.

Product Summary and Explanation

Streptococcus mitis, *Streptococcus salivarius*, and *Enterococcus* spp. are part of 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, bacteremia, and can colonize the skin and mucous membranes.²

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide carbon, nitrogen, and amino acids used for general growth requirements in Mitis Salivarius Agar. Sucrose and Dextrose are carbohydrate sources. Dipotassium Phosphate is the buffering agent. Trypan Blue is absorbed by the colonies, producing a blue color. Crystal Violet and Potassium Tellurite inhibit most Gram-negative bacilli and Gram-positive bacteria except streptococci. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Animal Tissue.....	5 g
Sucrose.....	50 g
Dextrose.....	1 g
Dipotassium Phosphate.....	4 g
Trypan Blue.....	0.075 g
Crystal Violet.....	0.0008 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

1% Potassium Tellurite, 1 mL

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Inhalation of powder may cause respiratory irritation.

Directions

1. Suspend 90 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool the sterile medium to 50 - 60°C and aseptically add 1 mL of a 1% filter sterilized potassium tellurite solution.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light blue-beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and deep royal blue.

Expected Cultural Response: Cultural response on Mitis Salivarius Agar, enriched with 1% potassium tellurite solution at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---
<i>Streptococcus mitis</i> ATCC® 9811	growth	blue colonies
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	blue colonies
<i>Streptococcus salivarius</i> ATCC® 13419	growth	blue "gum drop" colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

S. mitis produces small 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* spp. form dark blue or black, shiny, slightly raised, 1 - 2 mm colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. If coliforms grow on the medium, they produce brown colonies.
3. Molds will grow on the medium after two days incubation.
4. *Erysipelothrix rhusiopathiae* produces colorless, circular, convex colonies.
5. Beta-hemolytic streptococci produce colonies that resemble *S. mitis*.

Packaging

Mitis Salivarius Agar	Code No.	7277A	500 g
		7277B	2 kg
		7277C	10 kg

References

1. **Facklam, R. R., and J. A. Washington II.** 1991. *Streptococcus* and related catalase-negative gram-positive cocci. p. 238-257. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (eds.). Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
2. **Facklam, R. R., and D. F. Sahn.** 1995. *Enterococcus*, p. 308-314. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Chapman, G. H.** 1944. The isolation of streptococci from mixed cultures. J. Bacteriol. **48**:113.
4. **Chapman, G. H.** 1946. The isolation and testing of fecal streptococci. Am. J. Dig. Dis. **13**:105.
5. **Chapman, G. H.** 1947. Relationship of nonhemolytic and viridans streptococci in man. Trans. N. Y. Acad. Sci. **10**:45.
6. **MacFaddin, J. F.** 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol. 1 Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MOTILITY TEST AGAR (7247)

Intended Use

Motility Test Agar is used for the differentiation of microorganisms on the basis of motility.

Product Summary and Explanation

In 1936, Tittsler and Sandholzer reported using a semisolid agar for the detection of bacterial motility.¹ Motility Test Agar is a modification of this formulation.

Bacterial motility is observed macroscopically by a diffuse zone of growth spreading from the line of inoculation. Certain species of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points appearing as nodular outgrowths along the stab. Tittsler and Sandholzer reported tubes incubated for one day gave identical results with the hanging drop method, and incubation for two days permitted demonstration of motility in an additional 4% of cultures tested.¹

Motility Test Agar is recommended for the detection of microbial motility in food and dairy standard methods.²⁻⁴

Principles of the Procedure

The nitrogen, carbon, and vitamin sources are provided by Enzymatic Digest of Gelatin and Beef Extract in Motility Test Agar. Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent used at a low concentration.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Beef Extract	3 g
Sodium Chloride	5 g
Agar	4 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system and skin.

Directions

1. Suspend 22 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear to trace hazy and yellow-beige.

Expected Cultural Response: Cultural response in Motility Test Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions (Motility)
<i>Escherichia coli</i> ATCC® 25922	growth	positive
<i>Salmonella choleraesuis</i> ATCC® 13076	growth	positive
<i>Staphylococcus aureus</i> ATCC® 25923	growth	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate tubes by stabbing through center of the medium with inoculating needle to approximately one-half the depth of the medium. Incubate at the proper temperature for the organism under consideration and examine at 18 – 48 hours. If negative, continue incubation at 22 - 25°C for an additional 5 days.

Results

Motility is observed visually by diffuse growth spreading from the line of inoculation. Certain strains of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular growths along the stab line. Non-motile organisms grow only along the line of inoculation.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Many organisms fail to grow deep in semisolid media, inoculating pour plates may be advantageous.⁵
2. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Motility Test Agar	Code No.	7247A	500 g
		7247B	2 kg
		7247C	10 kg

References

1. **Tittsler, R. P., and L. A. Sandholzer.** 1936. The use of semi-solid agar for the detection of bacterial motility. J. Bacteriol. **31**:575-580.
2. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
3. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 110-114. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MRSA AGAR BASE (7420)

Intended Use

MRSA Agar Base is used with added oxacillin (6 mg/L) in the preparation of MRSA Agar. MRSA Agar is used as a screening medium for the determination of methicillin resistance and oxacillin resistance in *Staphylococcus aureus*.

Product Summary and Explanation

Methicillin-resistant strains of *S. aureus* (MRSA) were first recognized in the 1980's as a major clinical and epidemiological problem.¹ Hospitals are still facing this problem today. MRSA Agar was developed to detect the presence of the *mecA* gene (classic resistance) in *S. aureus*.²

Principles of the Procedure

MRSA Agar Base is composed of Mueller Hinton Agar and Sodium Chloride. Mueller Hinton Agar is made up of Beef Extract and Acid Hydrolysate of Casein, providing nitrogen, vitamins, carbon, and amino acids. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. The high concentration of Sodium Chloride enhances growth of *S. aureus*. Oxacillin is added to determine if the particular strain of *S. aureus* is oxacillin resistant.

Formula / Liter

Mueller Hinton Agar 38 g

Sodium Chloride 40 g

Final pH: 7.3 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobial Additive

Oxacillin 6 mg/10 mL

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 78 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. After cooling to 45 - 50°C aseptically add 10 mL of a filter sterilized solution of oxacillin (6mg/10mL purified water).

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium is hazy, and yellow beige.

Expected Cultural Response: Cultural response at 35°C after 24 hours incubation.

Microorganism	Response	
	Without Oxacillin	With Oxacillin
<i>Staphylococcus aureus</i> ATCC® 25923 (susceptible)	growth	no growth
<i>Staphylococcus aureus</i> ATCC® 43300 (resistant)	growth	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate MRSA Agar plates with 10 mcl of a 1:100 dilution of a 0.5 MacFarland standardized suspension of the strain of *S. aureus* to be tested. Incubate plates for 24 hours at 35°C and examine for any evidence of growth.

Results

The presence of growth indicates oxacillin and methicillin resistance. Lack of growth indicates that the strain lacks the *mecA* resistance gene. For a complete discussion on oxacillin resistance screening, please consult appropriate references.^{2,3}

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Packaging

MRSA Agar Base	Code No.	7420A	500 g
		7420B	2 kg
		7420C	10 kg

References

1. **Murray, P. R., E. J. Baron, and M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 4th ed. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Villanova, PA.
3. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests. 6th ed. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Villanova, PA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MR-VP BROTH (7237)

Intended Use

MR-VP Broth is used for the differentiation of microorganisms on the basis of acid or acetylmethyl carbinol production (MR-VP reaction).

Product Summary and Explanation

In 1915, Clark and Lubs demonstrated that colon-aerogenes family of bacteria could be divided into two groups based on their action in a peptone and dextrose medium.¹ When tested with the pH indicator methyl red, the “coli” group produced high acidity while the “aerogenes” group produced a less acid reaction. The test to detect high-acid end products is known as the Methyl Red (MR) test. The test to detect less-acid end products is based on the procedure described by Voges and Proskauer in 1898.² A color reaction occurs when certain cultures, incubated in a medium containing peptone and dextrose, are treated with potassium hydroxide and exposed to air. This reaction detects the formation of acetylmethylcarbinol, known as the Voges-Proskauer (VP) test.

The MR and VP tests appear in the identification scheme for *Enterobacteriaceae*, important isolates in clinical microbiology³ and food and dairy microbiology testing.^{4,5} MR-VP Broth is also known as Methyl Red-Voges-Proskauer Medium.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are carbon, nitrogen, and vitamin sources used for general growth requirements in MR-VP Broth. Dextrose is the fermentable carbohydrate. Potassium Phosphate is a buffering agent. Members of *Enterobacteriaceae* convert glucose to pyruvate by the Embden-Meyerhof pathway. Some bacteria metabolize pyruvate by the mixed acid pathway and produce acidic end products (pH < 4.4), such as lactic, acetic, and formic acids. Other bacteria metabolize pyruvate by the butyleneglycol pathway and produce neutral end products (pH > 6.0), one of which is acetoin (acetylmethylcarbinol). In the MR test the pH indicator, methyl red, detects acidic end products.⁶ In the VP test, acetoin is oxidized in the presence of oxygen and potassium hydroxide (KOH) to diacetyl, producing a red color.⁶ The addition of naphthol before KOH enhances the sensitivity of the test.⁶

Formula / Liter

Enzymatic Digest of Casein	3.5 g
Enzymatic Digest of Animal Tissue.....	3.5 g
Dextrose.....	5 g
Potassium Phosphate	5 g
Final pH: 6.9 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Dissolve 17 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is yellow gold to amber and clear.

Expected Cultural Response: Cultural response in MR-VP Broth after 2 days at 35°C incubation for Methyl Red test and 2 days at 35°C incubation for Voges-Proskauer test.

Microorganism	Response	Reactions	
		MR	VP
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	negative	positive
<i>Escherichia coli</i> ATCC® 25922	growth	positive	negative
<i>Proteus vulgaris</i> ATCC® 13315	growth	positive	negative
<i>Serratia marcescens</i> ATCC® 8100	growth	negative	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate MR-VP Broth with growth from a single colony. Incubate at 35 ± 2°C for 48 hours. Proceed with Methyl Red or Voges-Proskauer test.

Methyl Red Test

Transfer 2.5 mL of the MR-VP Broth culture to a tube (13 x 100mm). Add 5 drops of Methyl Red and observe for a color change.

Voges-Proskauer Test

Transfer 2.5 mL of the MR-VP Broth culture to a tube (13 x 100mm). Add 0.3 mL (6 drops) of Voges-Proskauer Reagent A (5% α-naphthol). Add 0.1 mL (2 drops) of Voges-Proskauer Reagent B (40% KOH). Gently agitate the tube and let stand for 10 – 15 minutes. Observe for a color change.

Results

Methyl Red (MR) Test: Positive – bright red color; Negative – yellow-orange color.

Note: If the test is negative continue to incubate the broth without added reagents, repeat the test after an additional 18 – 24 hours incubation.

Voges-Proskauer (VP) Test: Positive – red color, Negative – no red color.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within *Enterobacteriaceae*.

Packaging

MR-VP Broth	Code No.	7237A	500 g
		7237B	2 kg
		7237C	10 kg

References

1. **Clark, W. M., and H. A. Lubs.** 1915. The differentiation of bacteria of the colon-aerogenes family by the use of indicators. J. Infect. Dis. 17:160-173.
2. **Voges, O., and B. Proskauer.** 1898. Z. Hyg. 28:20-22.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
4. **Vanderzant, C. and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Marshall, R. T. (ed.).** Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MUELLER HINTON AGAR (7101)

Intended Use

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. This formula conforms to National Committee for Clinical Laboratory Standards (NCCLS).¹

Product Summary and Explanation

Mueller Hinton Agar is based on the formula recommended by Mueller and Hinton² for the primary isolation of *Neisseria species*. Mueller and Hinton selected pea meal extract agar as a simple transparent medium containing heat stable ingredients.³ During their modification, starch replaced the growth-promoting properties of pea extract, acting as a “protective colloid” against toxic substances.

Bauer, Kirby, Sherris and Tuck⁴ recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disk of high concentration. This unsupplemented medium has been selected by the National Committee for Clinical Laboratory Standards (NCCLS)¹ for several reasons:⁵ this medium is low in sulfonamide, trimethoprim and tetracycline inhibitors, provides satisfactory growth of most non-fastidious pathogens and demonstrates batch-to-batch reproducibility.

Mueller Hinton Agar is often abbreviated as M-H Agar, and complies with requirements of the World Health Organization.⁵ Mueller Hinton Agar is specified in FDA Bacteriological Analytical Manual⁶ for food testing, and procedures commonly performed on aerobic and facultatively anaerobic bacteria.⁷ A variety of supplements can be added to Mueller Hinton Agar, including 5% defibrinated sheep or horse blood, 1% growth supplement and 2% sodium chloride.

Principles of the Procedure

Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent.

A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Formula / Liter

Beef Extract	2 g
Acid Hydrolysate of Casein	17.5 g
Starch.....	1.5 g
Agar	17 g

Final pH 7.3 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 38 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. OPTIONAL: Supplement as appropriate. Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth. Allow to cool to room temperature.
5. Check prepared Mueller Hinton Agar to ensure the final pH is 7.3 ± 0.1 at 25°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is slightly opalescent with no significant precipitation, and light to medium amber.

Expected Cultural Response: Prepare, inoculate and dispense antibiotic disks following the procedure described by NCCLS.^{1,8,9} The cultures listed should have middle range zone sizes of the concentration tested.⁸

Microorganism	Response & Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	growth; zone diameters within published specifications
<i>Escherichia coli</i> ATCC® 25922	growth; zone diameters within published specifications
<i>Escherichia coli</i> ATCC® 35218	growth; zone diameters within published specifications
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth; zone diameters within published specifications
<i>Staphylococcus aureus</i> ATCC® 25923	growth; zone diameters within published specifications
<i>Staphylococcus aureus</i> ATCC® 43300	growth; zone diameters within published specifications

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on antimicrobial susceptibility testing, refer to procedures outlined in appropriate references.

Results

Refer to appropriate documents for correct zone sizes.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Numerous factors can affect results: inoculum size, rate of growth, medium formulation and pH. Strict adherence to protocol is required to ensure reliable results.⁹
3. Drug inactivation may result from the prolonged incubation times required by slow growers.¹⁰
4. Variation in the concentration of divalent cations, primarily calcium and magnesium affects result of aminoglycoside, tetracycline, and colistin test with *P. aeruginosa* isolates.⁷

Packaging

Mueller Hinton Agar	Code No.	7101A	500 g
		7101B	2 kg
		7101C	10 kg

References

1. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, PA.
2. **Mueller, J. H., and J. Hinton.** 1941. A protein-free medium for primary isolation of gonococcus and meningococcus. Proc. Soc. Exp. Biol. Med. **48**:3330-333.
3. **Gordon and Hine.** 1916. Br. Med. J. **678**.
4. **Bauer, A. L., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**:493-496.
5. **World Health Organization.** 1961. Standardization of methods for conducting microbic sensitivity tests. Technical Report Series No. 210, Geneva.
6. **Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
7. **Wood, G. L., and J. A. Washington.** 1995. Antibacterial susceptibility tests: dilution and disk diffusion methods, p. 1327-1341. In Murray, P.R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. **National Committee for Clinical Laboratory Standards.** 1996. Protocols for evaluating dehydrated; App. Standard. Wayne PA.
9. **National Committee for Clinical Laboratory Standards.** 1999. M100-S9. Performance Standards for Antimicrobial Susceptibility Testing; Ninth Informational Supplement. Wayne, PA.
10. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MYCOBIOTIC AGAR (7419)

Intended Use

Mycobiotic Agar is used for the selective isolation of pathogenic fungi from clinical materials.

Product Summary and Explanation

The value of selective media for initial cultivation of pathogenic fungi has been demonstrated by numerous investigators.¹⁻³ Historically, media for fungi generally relied on an acid pH to make the media less suitable for growth of many bacteria.⁴ Recently developed media use neutral or slightly alkaline reactions, antibiotics, bile salts, and dyes as selective agents against bacteria.^{5,6} Mycobiotic Agar is an excellent basal medium and antifungal agents, cycloheximide and chloramphenicol, are added to study their affect on fungi. This medium is proven useful in the isolation of dermatophytes and other pathogenic fungi from clinical specimens.⁷

Georg recommends the use of Mycobiotic Agar exclusively for isolating dermatophytes (dermatophytes are not sensitive to cycloheximide or chloramphenicol) and in parallel to media without antibiotics for isolating fungi which cause systemic disease.⁸

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Soybean Meal in Mycobiotic Agar. Dextrose is the carbohydrate source. Cycloheximide suppresses the growth of saprophytic fungi. Chloramphenicol inhibits bacterial growth. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Soybean Meal	10 g
Dextrose.....	10 g
Agar	15 g
Cycloheximide.....	0.5 g
Chloramphenicol.....	0.05 g

Final pH: 6.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. VERY TOXIC. Toxic by inhalation and contact with skin.

Directions

1. Suspend 35.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 10 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and light to medium yellow.

Expected Cultural Response: Cultural response on Mycobiotic Agar at 25 - 30°C after 2 - 7 days of incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	partial to complete inhibition
<i>Candida albicans</i> ATCC® 10231	growth
<i>Microsporum audouinii</i> ATCC® 42558	growth
<i>Penicillium roquefortii</i> ATCC® 10110	inhibited
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and identification of fungi.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Non-selective fungal media should be used concurrently with selective media when isolating fungi due to the sensitivity of some strains to cycloheximide and chloramphenicol.⁸

Packaging

Mycobiotic Agar	Code No. 7419A	500 g
	7419B	2 kg
	7419C	10 kg

References

1. **Am. J. Public Health.** 1951. **41**:292.
2. **Bull. D. Inst. Sieroteropl., Melan.** 1926. **5**:173.
3. **Am. Rev. Resp. Dis.** 1967. **95**:1041.
4. **Am. J. Clin. Pathol.** 1951. **21**:684.
5. **Am. J. Clin. Pathol.** 1954. **24**:621.
6. **Rev. Latinoam Microbiol.** 1958. **1**:125.
7. **Land, G. A.** 1992. Culture media. *In* H. D. Isenberg, (ed.). Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
8. **Georg, L. K., E. S. McDonough, L. Ajello, and S. Brinkman.** 1960. In vitro effects of antibiotics on yeast phase of *Blastomyces dermatitidis* and other fungi. *J. Lab. & Clin. Med.* **55**:116-19.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

MYCOLOGICAL AGAR (7309)

Intended Use

Mycological Agar is used for the cultivation of fungi.

Product Summary and Explanation

The value of selective media for initial cultivation of pathogenic fungi has been demonstrated by numerous investigators.¹⁻³ Historically, media for fungi generally relied on an acid pH to make the media less suitable for growth of many bacteria.⁴ Recently, media have been developed using neutral or slightly alkaline reactions, antibiotics, bile salts, and dyes as selective agents against bacteria.^{5,6} Mycological Agar is an excellent basal medium and antifungal agents may be added to study their affect on fungi.

Mycological Agar is prepared according to the formulation suggested by Huppert and Walker.⁷ Mycological Agar has a lower dextrose content than Sabouraud Dextrose Agar, and recommended for the isolation and cultivation of fungi from clinical specimens, foods,⁸ and cosmetics.⁹ This medium may be adjusted to pH 4.0 after autoclaving by adding sterile lactic acid or acetic acid.

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Soybean Meal in Mycological Agar. Dextrose is the carbohydrate source. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Soybean Meal 10 g
Dextrose..... 10 g
Agar 16 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 36 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, and yellowish- tan in color.

Expected Cultural Response: Cultural response on Mycological Agar at 25- 30 °C after 2-7 days incubation.

Microorganism	Response	Reactions
<i>Aspergillus niger</i> ATCC® 16404	growth	white- cottony to wooly and black mycelium; powdery spores
<i>Candida albicans</i> ATCC® 10231	growth	off-white, pasty
<i>Mycosporum canis</i> ATCC® 36299	growth	white velvety to cottony mycelium

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and identification of fungi.

Results

Refer to appropriate references and procedures for results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Mycological Agar	Code No.	7309A	500 g
		7309B	2 kg
		7309C	10 kg

References

1. **Am. J. Public Health.** 1951. **41**:292.
2. **Bull. D. Inst. Sieroteropl., Melan.** 1926. **5**:173.
3. **Am. Rev. Resp. Dis.** 1967. **95**:1041.
4. **Am. J. Clin. Pathol.** 1951. **21**:684.
5. **Am. J. Clin. Pathol.** 1954. **24**:621.
6. **Rev. Latinoam Microbiol.** 1958. **1**:125.
7. **Huppert, M., and L. J. Walker.** 1958. The selective and differential effects of cycloheximide on many strains of *Coccidioides immitis*. *Am. J. Clin. Pathol.* **29**:291.
8. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 65-68. Williams & Wilkins, Baltimore, MD.
9. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr.** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

NUTRIENT AGAR (7145)

Intended Use

Nutrient Agar is used for the cultivation of a wide variety of microorganisms.

Product Summary and Explanation

In the early 1900's, the American Public Health Association (APHA) suggested the formula of Nutrient Agar as a standard culture medium used in water testing.¹ Nutrient Agar continues to be a widely used general purpose medium for growing nonfastidious microorganisms. If required, enrichments can be added to this medium. Nutrient Agar, modified by incorporating 4-methylumbelliferyl- β -D-glucuronide (MUG), is used for fluorogenic detection of *Escherichia coli*.²

Nutrient Agar meets APHA and Association of Official Analytical Chemists (AOAC) standard methods.^{2,3} Nutrient Agar is specified in many standard methods procedures for the examination of food, dairy products, water, and other materials.²⁻⁵

Principles of the Procedure

The nitrogen, carbon, vitamins, and amino acids in Nutrient Agar are provided by Enzymatic Digest of Gelatin and Beef Extract. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin 5 g

Beef Extract 3 g

Agar 15 g

Final pH: 6.8 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 23 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace hazy and light amber.

Expected Cultural Response: Cultural response on Nutrient Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate medium with isolated colonies or a loopful of pure culture from broth. Streak for isolation.
2. Incubate aerobically at 35°C for 18 – 24 hours or longer if necessary.

Results

Good growth of nonfastidious organisms on Nutrient Agar will appear as translucent colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Nutrient Agar	Code No.	7145A	500 g
		7145B	2 kg
		7145C	10 kg

References

1. **American Public Health Association.** 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

NUTRIENT AGAR 1.5% (7286)

Intended Use

Nutrient Broth 1.5% is used for the cultivation of a wide variety of microorganisms.

Product Summary and Explanation

In the early 1900's, the American Public Health Association (APHA) suggested the formula of Nutrient Agar as a standard culture medium used in water testing.¹ Nutrient Agar is specified in many standard method procedures.²⁻⁶ Nutrient Agar 1.5% is a modification of Nutrient Agar. Nutrient Agar 1.5% is a general purpose medium, with a slightly alkaline pH. This medium contains 0.8% sodium chloride and can be used as a base for enrichment with blood, ascitic fluid, or other supplements for cultivating fastidious microorganisms.

Principles of the Procedure

The nitrogen, carbon, vitamins, and amino acids are provided by Enzymatic Digest of Gelatin and Beef Extract. Sodium Chloride maintains the osmotic balance of the medium so red blood cells will not rupture when the medium is supplemented with blood.² Agar is the solidifying agent.

Formula / Liter

Beef Extract	3 g
Enzymatic Digest of Gelatin	5 g
Sodium Chloride	8 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 31 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and beige.

Expected Cultural Response: Cultural response on Nutrient Agar 1.5% at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of aerobic and anaerobic microorganisms, refer to appropriate references.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Nutrient Agar 1.5%	Code No.	7286A	500 g
		7286B	2 kg
		7286C	10 kg

References

1. **American Public Health Association.** 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
6. **Bacteriological Analytical Manual.** 1995. 8th ed. Association of Official Analytical Chemists. Gaithersburg, MD.

Technical Information

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NUTRIENT BROTH (7146)

Intended Use

Nutrient Broth is used for the cultivation of a wide variety of microorganisms.

Product Summary and Explanation

In the early 1900's, the American Public Health Association (APHA) suggested the formula of Nutrient Agar as a standard culture medium used in water testing.¹ Nutrient Broth is the same formulation as Nutrient Agar, only Agar has been omitted.

Nutrient Broth is used as a pre-enrichment medium when testing certain foods and dairy products for *Salmonella* spp. In dried or processed foods, salmonellae may be sublethally injured and in low numbers. The presence of other bacteria and food sample components may hinder growth and recovery of *Salmonella* spp. Pre-enrichment in a nonselective medium such as Nutrient Broth allows for cell damage repair, dilutes toxic or inhibitory substances, and provides a nutritional advantage to *Salmonella* over other bacteria.²

Nutrient Broth is included in many standard methods procedures for testing food, dairy products, and other materials.²⁻⁶

Principles of the Procedure

The nitrogen, carbon, vitamins, and amino acids in Nutrient Broth are provided by Enzymatic Digest of Gelatin and Beef Extract.

Formula / Liter

Enzymatic Digest of Gelatin 5 g

Beef Extract 3 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 8 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear and yellow to gold.

Expected Cultural Response: Cultural response in Nutrient Broth at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Direct:

1. Inoculate broth with specimen.
2. Incubate aerobically at 35°C for 18 – 24 hours or longer if necessary.

Pre-enrichment:

1. Mix 25 g of the sample with 225 mL of Nutrient Broth.
2. Incubate at 35°C for 18 – 24 hours.
3. Transfer a portion to one or more selective enrichment broths.

Note: Refer to appropriate references for specific recommendations when testing certain foods and dairy products for *Salmonella* spp.

Results

Turbidity indicates good growth.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Nutrient Broth	Code No.	7146A	500 g
		7146B	2 kg
		7146C	10 kg

References

1. **American Public Health Association.** 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
6. **Bacteriological Analytical Manual.** 1995. 8th ed. Association of Official Analytical Chemists. Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

NUTRIENT GELATIN (7471)

Intended Use

Nutrient Gelatin is used for the differentiation of microorganisms on the basis of gelatinase production.

Product Summary and Explanation

Gelatin was the first gelling agent used to solidify culture media. The advantages of solid media over liquid media include isolation of pure cultures and the ability to perform plate counts. The disadvantages of gelatin include incubation at 20°C, a temperature that is lower than optimum for growing many microorganisms, and the fact that many organisms metabolize (liquefy) gelatin. Agar later replaced gelatin as a solidifying agent.

Identifying fermentative and non-fermentative gram-negative bacilli include testing for gelatin liquefaction. If the proteolytic enzyme gelatinase is present, gelatin is hydrolyzed and loses its gelling characteristic.¹ Edwards and Ewing include this test in the differentiation scheme for *Enterobacteriaceae*.² Procedures for performing the standard tube method for gelatin liquefaction are available.²⁻⁴

Principles of the Procedure

The nitrogen, carbon, vitamins, and amino acids are provided by Enzymatic Digest of Gelatin and Beef Extract for general growth requirements in Nutrient Gelatin. Gelatin is the substrate for determining if microorganisms elaborate the proteolytic enzyme to hydrolyze (liquefy) gelatin.

Formula / Liter

Enzymatic Digest of Gelatin 5 g
Beef Extract 3 g
Gelatin..... 120 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 128 g of the medium in one liter of purified water.
2. Heat with frequent agitation to 50°C to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is light yellow to yellowish beige and trace to slightly hazy.

Expected Cultural Response: Cultural response in Nutrient Gelatin at 35°C after 2 - 7 days incubation.

Microorganism	Response	Reactions (Gelatinase)
<i>Bacillus subtilis</i> ATCC® 9372	growth	positive
<i>Clostridium perfringens</i> ATCC® 13124	growth	positive
<i>Escherichia coli</i> ATCC® 25922	growth	negative
<i>Staphylococcus aureus</i> ATCC® 25923	growth	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure¹

1. Using a sterile inoculating needle, touch several similar, well-isolated colonies on agar and stab directly down the center of the tube to approximately 10 mm from the bottom.
2. Incubate at $35 \pm 2^{\circ}\text{C}$ for 24 - 48 hours. Incubate uninoculated control tube with the test. Incubation may be extended to 14 days for some organisms.
3. Examine at various intervals. Transfer the tubes to a refrigerator or ice bath. Do not shake the tubes when transferring from incubator to refrigerator. Gently invert the chilled tubes to test for solidity.

Results

Positive: Medium remains liquefied after refrigeration.

Negative: Medium becomes solid after refrigeration.

Uninoculated control tube: Medium becomes solid after refrigeration.

Storage

Store sealed bottle containing the dehydrated medium at $2 - 30^{\circ}\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Use this method for detecting gelatinase only if the identification procedure permits incubation beyond 48 hours.
3. 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 uninoculated tube in the test procedure for comparison.
4. Growth and liquefaction frequently occur only at the tube surface. To prevent a false-negative interpretation, handle tubes carefully when warm so liquefied gelatin remains at the surface of the tube.

Packaging

Nutrient Gelatin	Code No.	7471A	500 g
		7471B	2 kg
		7471C	10 kg

References

1. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, Sup. 1. American Society for Microbiology, Washington, D.C.
2. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co. Inc. New York, NY.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

ORANGE SERUM AGAR (7587)

Intended Use

Orange Serum Agar is used for the cultivation of aciduric microorganisms associated with spoilage of products.

Product 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 1950's, 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 Murdock, Folinazzo, and Troy³ found Orange Serum Agar, pH 5.4 to be a suitable medium for growing *Leuconostoc*, *Lactobacillus*, and yeasts.

Orange Serum Agar is recommended for examining fruit beverages.¹

Principles of the Procedure

Enzymatic Digest of Casein provides carbon and nitrogen sources for general growth requirements. Orange Serum provides the acid environment favorable to recovering acid-tolerant microorganisms. Yeast Extract supplies B-complex vitamins that stimulate growth. Dextrose is the fermentable carbohydrate. Potassium Phosphate is a buffering agent. Agar is the solidifying agent.

Formula / Liter

Orange Serum.....	200 mL
Yeast Extract.....	3 g
Enzymatic Digest of Casein	10 g
Dextrose.....	4 g
Potassium Phosphate	2.5 g
Agar	17 g

Final pH: 5.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 45.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is slightly hazy and light to medium amber.

Expected Cultural Response: Cultural response on Orange Serum Agar at 35°C after 40 - 48 hours incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	good growth
<i>Lactobacillus casei</i> ATCC® 393	good growth
<i>Lactobacillus fermentum</i> ATCC® 9338	good growth
<i>Lactobacillus plantarum</i> ATCC® 8014	good growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. For plate count method, prepare serial 10-fold dilutions of the test material.
2. Add 1 mL of test sample to a petri dish.
3. Add 18 - 20 mL of sterile, 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.

Results

Record colony morphology for each type of growth.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Orange Serum Agar	Code No.	7587A	500 g
		7587B	2 kg
		7587C	10 kg

References

1. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C
2. **Hays, G. L.** 1951. The isolation, cultivation and identification of organisms which have caused spoilage in frozen concentrated orange juice. Proc. Fla. State Hortic. Soc. **54**:135-137.
3. **Murdock, D. I., J. F. Folinazzo, and V. S. Troy.** 1952. Evaluation of plating media for citrus concentrates. Food Technol. **6**:181-185.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

OXBILE (OXGALL) (7216)

Intended Use

Oxbile (Oxgall) is dehydrated bile for use in preparing microbiological culture media.

Product Summary and Explanation

Oxbile is manufactured from large quantities of fresh bile by rapid evaporation of the water content. Bile is composed of fatty acids, bile acids, inorganic salts, sulfates, bile pigments, cholesterol, mucin, lecithin, glycuronicacids, porphyrins, and urea. The use of Oxbile insures a regular supply of bile, and uniformity impossible to obtain with fresh materials.

Oxbile is dehydrated fresh bile and prepared specifically for differentiation of bile tolerant microorganisms. A 10% solution of dehydrated bile is equivalent to a fresh bile solution. It is usually incorporated into media e.g., Bile Esculin Agar and Brilliant Green Bile Agar, used for the determination of enteric pathogens. Oxbile is also found in Littman Agar, a selective fungal medium.

Principles of the Procedure

Oxbile is used as a selective agent for the isolation of gram-negative microorganisms, inhibiting gram-positive bacteria. The major composition of Oxbile is taurocholic and glycocholic acids.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and straw to beige in color.

Prepared Appearance (2.0% wt/vol): Prepared medium is clear, amber, with no or a light precipitate.

pH (2% Solution at 25°C): 7.0 - 8.5

Expected Cultural Response: Cultural response in Brilliant Green Bile Broth, 2% after incubation at 35°C for 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth with gas
<i>Staphylococcus aureus</i> ATCC 25923	marked to complete inhibition

Test Procedure

Refer to appropriate references for specific procedures using Oxbile. For a complete discussion on enteric pathogens, refer to procedures outlined in the references.^{1,2}

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Oxbile at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Oxbile should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to Oxbile in its intact container when stored as directed.

Packaging**Ox bile**

Code No.	7216A	500 g
	7216B	2 kg
	7216C	10 kg

References

1. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D.C.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

OXFORD LISTERIA AGAR BASE (7428)

Intended Use

Oxford Listeria Agar Base is used with antimicrobics for the selective isolation of *Listeria* spp.

Product Summary and Explanation

Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann,¹ is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant woman.² Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission included turkey frankfurters,⁴ coleslaw, pasteurized milk, Mexican style cheese, and pate'. *Listeria* spp. are ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, and river water.⁵

Oxford Listeria Agar Base is prepared according to the formulation of Curtis et al.⁶ *Listeria* spp. grow over a pH range of 5.0 - 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, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

Principles of the Procedure

Columbia Blood Agar Base contains Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Yeast Enriched Peptone providing nitrogen, carbon, amino acids, and vitamins. 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.⁸ Selectivity is provided by the presence of Lithium Chloride. The high salt tolerance of *Listeria* is used as a means to markedly inhibit growth of enterococci. Agar is the solidifying agent.

Selectivity is increased by adding various antimicrobial agents to the base. Incorporating these antimicrobial agents into Oxford Listeria Agar 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 formulation⁶ and the Modified Oxford Medium formulation.⁹ The Oxford Medium formulation contain cycloheximide, colistin sulfate, acriflavin, cefotetan, and fosfomycin. The Modified Oxford Medium formulation contains moxalactam and colistin sulfate.

Modified Oxford Medium is recommended for isolating and identifying *Listeria monocytogenes* from processed meat and poultry products.⁹ Oxford Medium is recommended for isolating *Listeria* from enrichment broth cultures.¹⁰

Formula / Liter

Columbia Blood Agar Base.....	39 g
Esculin	1 g
Ferric Ammonium Citrate.....	0.5 g
Lithium Chloride	15 g
Agar	2 g
Final pH: 7.2 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobics

Oxford Medium

Acriflavin	5 mg
Cefotetan	2 mg
Colistin Sulfate	20 mg
Cycloheximide	400 mg
Fosfomycin	10 mg

Precautions

1. For Laboratory Use.
2. **Oxford Medium Base**
HARMFUL. Harmful if swallowed, inhaled, or absorbed through the skin.

Modified Oxford Medium

Colistin Sulfate	10 mg
Moxalactam	20 mg

Directions

1. Suspend 57.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 10 minutes. Cool to 45 - 50°C.

Oxford Medium

Aseptically add a filtered sterilized aqueous solution of 5 mg acriflavin, 2 mg cefotetan, 20 mg colistin sulfate, 400 mg cycloheximide, and 10 mg fosfomycin.

Modified Oxford Medium

Aseptically add a filtered sterilized aqueous solution of 10 mg colistin sulfate and 20 mg moxalactam.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium amber and slightly hazy.

Expected Cultural Response: Cultural response in Oxford Listeria Agar Base and Modified Oxford Listeria Agar at 35°C after 24 - 48 hours incubation.

Microorganism	Response	
	Oxford	Modified Oxford
<i>Escherichia coli</i> ATCC® 25922	inhibited	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	good growth	good growth
<i>Listeria monocytogenes</i> ATCC® 19111	good growth	good growth
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

The USDA method⁹ 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 Method¹⁰ involves adding 25 mL of liquid or 25 g of solid material to 225 mL Listeria Enrichment Broth and incubating at 30°C for two days. After enrichment, the broth is plated onto Oxford Medium. For further information consult appropriate references.^{7, 9, 10, 11}

Results

Select esculin-positive colonies and confirm their identity through biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification. For additional information, refer to appropriate references.^{7, 9-11}

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. An identification of *L. monocytogenes* must be confirmed through biochemical and serological testing.¹¹
2. Poor growth and a weak esculin reaction maybe seen after 40 hours incubation for some enterococci.

Packaging

Oxford Listeria Agar Base	Code No.	7428A	500 g
		7428B	2 kg
		7428C	10 kg

References

1. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bacteriol. 29:407-439.
2. Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen and refrigerated ground beef. J. Food Prot. 57:969-974.
3. Bremer, P. J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. 58:604-608.
4. Graud, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. 55:4-7.
5. Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. 58: 244-250.
6. Curtis, G. D. W., R. G. Mitchell, A. F. King, and J. Emma. 1989. A selective differential medium for the isolation of *Listeria monocytogenes*. Appl. Microbiol. 8:95-98.
7. Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. Fraser, J., and W. Sperber. 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. 51:762-765.
9. Lee, W. H., and D. McClain. 1989. Laboratory Communication No. 57 (revised May 24, 1989). U.S.D.A., F.S.I.S. Microbiology Division, Beltsville, MD.
10. U.S. Food and Drug Administration. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
11. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PANCREATIC DIGEST OF CASEIN (7179) (Peptone C)

Intended Use

Pancreatic Digest of Casein (Peptone C) is an enzymatic digest of casein for use in preparing microbiological culture media.

Product Summary and Explanation

Pancreatic Digest of Casein is recommended for preparing media where an enzymatic hydrolyzed casein is desired. Pancreatic Digest of Casein is used to support the growth of fastidious microorganisms. The high tryptophane content of Pancreatic Digest of Casein is valuable for use in detecting indole production. This media ingredient is biologically free of carbohydrates, and is recommended for fermentation studies. Nitrate reduction can also be determined using Pancreatic Digest of Casein.

Media used for the enumeration of coliforms in water use Pancreatic Digest of Casein as a nitrogen source. Pancreatic Digest of Casein is recommended for preparing media for sterility testing according to US Pharmacopeia XXIII (USP).¹ Several media containing Pancreatic Digest of Casein are specified in standard methods for multiple applications.²⁻⁴

Principles of the Procedure

Pancreatic Digest of Casein provides nitrogen, vitamins, minerals, and amino acids in prepared culture media. Casein is the main protein of milk, and a rich source of amino acid nitrogen.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and light beige.

Prepared Appearance (2% wt/ vol): Prepared medium is clear, light yellow with no or a light precipitate.

pH (2% Solution at 25°C): 6.6 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35°C after 18-24 hour incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to good growth

Test Procedure

Refer to appropriate references for specific procedures using Pancreatic Digest of Casein.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Pancreatic Digest of Casein at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Pancreatic Digest of Casein should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to Pancreatic Digest of Casein in its intact container when stored as directed.

Packaging

Pancreatic Digest of Casein (Peptone C)	Code No.	7179A	500 g
		7179B	2 kg
		7179C	10 kg

References

1. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PANCREATIC DIGEST OF GELATIN (7182) (Peptone G)

Intended Use

Pancreatic Digest of Gelatin (Peptone G) is an enzymatic digest of gelatin for use in preparing microbiological culture media.

Product Summary and Explanation

Pancreatic Digest of Gelatin is used as a media ingredient for fermentation studies, and alone to support the growth of non-fastidious microorganisms. Pancreatic Digest of Gelatin is deficient in carbohydrates, and distinguished by low cystine and tryptophan content.

Principles of the Procedure

Pancreatic Digest of Gelatin provides nitrogen, amino acids, vitamins, and carbon in microbiological culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and beige.

Prepared Appearance (2% wt/vol): Prepared medium is clear, pale to light yellow with no or a light precipitate.

pH (2% Solution at 25°C): 6.5 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35° C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	fair to good growth
<i>Staphylococcus aureus</i> ATCC 25923	poor to fair growth

Test Procedure

Refer to appropriate references for specific procedures using Pancreatic Digest of Gelatin.¹⁻⁴

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Pancreatic Digest of Gelatin at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Pancreatic Digest of Gelatin should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Pancreatic Digest of Gelatin in its intact container when stored as directed.

Packaging

Pancreatic Digest of Gelatin (Peptone G)	Code No.	7182A	500 g
		7182B	2 kg
		7182C	10 kg

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PAPAIC DIGEST OF SOYBEAN MEAL (7180) (Peptone S)

Intended Use

Papaic Digest of Soybean Meal (Peptone S) is an enzymatic digest of soybean meal for use in preparing microbiological culture media.

Product Summary and Explanation

Papaic Digest of Soybean Meal is an enzymatic hydrolysate of soybean meal prepared under controlled conditions for use in microbiological procedures. Papaic Digest of Soybean Meal is recommended for use in media for the cultivation of a large variety of organisms, including fungi and microbiological assay media. The nitrogen source in Papaic Digest of Soybean Meal contains naturally occurring, high concentrations of vitamins and carbohydrates of soybean. Due to its high carbohydrate content, Papaic Digest of Soybean Meal cannot be used for fermentation studies. This media ingredient produces clear solutions at culture media concentrations and neutral reactions after autoclaving.

Papaic Digest of Soybean Meal minimizes bovine spongiform encephalopathy (BSE) risk in vaccine production because of the plant origin of this product.

Principles of the Procedure

Papaic Digest of Soybean Meal provides nitrogen, vitamins, and minerals in prepared culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing beige.

Prepared Appearance (2% wt/vol): Prepared medium is clear, amber with no or a light precipitate.

pH (2% Solution at 25° C): 6.5 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35° C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to good growth

Test Procedure

Refer to appropriate references for specific procedures using Papaic Digest of Soybean Meal.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Papaic Digest of Soybean Meal at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Papaic Digest of Soybean Meal should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to Papaic Digest of Soybean Meal in its intact container when stored as directed.

Packaging

Papaic Digest of Soybean Meal (Peptone S)	Code No.	7180A	500 g
		7180B	2 kg
		7180C	10 kg

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PEPTIC DIGEST OF ANIMAL TISSUE (7181) (Peptone A)

Intended Use

Peptic Digest of Animal Tissue (Peptone A) is an enzymatic digest of animal tissue used in preparing microbiological culture media.

Product Summary and Explanation

Peptic Digest of Animal Tissue was developed as an alternate nitrogen source in dehydrated culture media to accommodate varying nutritional requirements. Fraser Broth and *Campylobacter* media contain Peptone A as a nitrogen and vitamin source.

Principles of the Procedure

Peptic Digest of Animal Tissue provides nitrogen, amino acids, vitamins, and carbon in microbiological culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and dark beige to tan.

Prepared Appearance (2% wt/vol): Prepared medium is clear, amber with no or a light precipitate.

pH (2% Solution at 25° C): 6.5 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35° C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to good growth

Test Procedure

Refer to appropriate references for specific procedures using Peptic Digest of Animal Tissue.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Peptic Digest of Animal Tissue at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Peptic Digest of Animal Tissue should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to Peptic Digest of Animal Tissue in intact container when stored as directed.

Packaging

Peptic Digest of Animal Tissue (Peptone A) Code No.	7181A	500 g
	7181B	2 kg
	7181C	10 kg

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PEPTONE WATER (7365)

Intended Use

Peptone Water is used for the cultivation of non-fastidious microorganisms, indole testing, and as a basal medium for carbohydrate fermentation studies.

Product Summary and Explanation

Peptone Water is a minimal growth medium. The formulation of Peptone Water permits cultivation of non-fastidious organisms.¹ This non-selective medium has been used as a basal medium for biochemical tests such as carbohydrate fermentation patterns and production of indole.^{1,2} A 1% final carbohydrate concentration with phenol red (0.018 g/L) indicator and a Durham tube can be used for fermentation studies.

Principles of the Procedure

Peptone Water contains Peptone as a source of carbon, nitrogen, vitamins, and minerals. Sodium Chloride maintains the osmotic balance.

Formula / Liter

Peptone..... 10 g

Sodium Chloride 5 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 15 g of the medium in one liter of purified water.
2. Heat with frequent agitation to obtain solution.
3. Autoclave at 121°C for 15 minutes.

If Determining Carbohydrate Fermentation Patterns

1. Add 1.8 mL of a 1% phenol red solution to 1 liter rehydrated Peptone Water. Mix thoroughly.
2. Dispense into test tubes containing inverted Durham tubes.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add a sufficient sterile carbohydrate solution to yield a 1% final concentration. Rotate each tube thoroughly to distribute the carbohydrate.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and tan.

Prepared Appearance: Prepared medium is gold to amber, clear to trace hazy, and may have none to light ppt.

Expected Cultural Response: Cultural response in Peptone Water after 24 hours incubation at 35°C.

Microorganism	Growth Response w/o Carbohydrates
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Carbohydrate Fermentation

Inoculate tubes with test organism. Incubate tubes at $35 \pm 2^{\circ}\text{C}$ for 18 - 48 hours. Observe for color change.

Indole Test

Using aseptic technique, suspend the commercially available Indole Test Strip 100 mm above the surface of a 24 or 48 hour culture. Incubate at 37°C for 5 - 30 minutes.

Results

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 Durham tube.

Indole Test

Observe for the formation of a violet color on the strip indicating a positive test for indole production.

Storage

Store sealed bottle containing the dehydrated medium at $2 - 30^{\circ}\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Peptone Water	Code No.	7365A	500 g
		7365B	2 kg
		7365C	10 kg

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 610-612. Williams & Wilkins, Baltimore, MD.
2. **Balows, A., W. J. Hausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (eds.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
3. **Finegold, S. M., and W. Martin.** 1982. Bailey and Scott's diagnostic microbiology, 6th ed. St. Louis.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PHENOL RED BROTH BASE (7148)

Intended Use

Phenol Red Broth Base is used with carbohydrates for the differentiation of microorganisms on the basis of carbohydrate fermentation reactions.

Product Summary and Explanation

The fermentative properties of bacteria are valuable criteria in their identification.^{1,2} A basal medium for determining the fermentation reactions of microorganisms must be capable of supporting the growth of test organisms and free from fermentable carbohydrates. Vera used a fermentation test medium employing the pH indicator phenol red and obtained accurate results.³

Phenol Red Broth Base is recommended for use to determine the ability of organisms to ferment various carbohydrates.⁴⁻⁶ Various fermentable substances may be added in any desired concentration. The concentration of carbohydrate generally employed for testing fermentation reactions of bacteria is 0.5 to 1%. Some investigators prefer to use 1% rather than 0.5% to ensure against reversion of the reaction due to depletion of the carbohydrate.

Principles of the Procedure

The nitrogen, amino acids, and carbon sources are provided by Enzymatic Digest of Casein in Phenol Red Broth Base. Sodium Chloride maintains the osmotic balance of the medium. Phenol Red serves as an indicator, turning from red-orange to yellow when acid is produced during fermentation of the added carbohydrates.

Formula / Liter

Enzymatic Digest of Casein 10 g
Sodium Chloride 5 g
Phenol Red 0.018 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Desired Carbohydrate, 5 – 10 g

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 15 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. If desired, add carbohydrates (5 – 10 grams). Autoclave at 121°C for 15 minutes.
4. Alternatively, filtered sterilized carbohydrate solutions may be added to the cooled sterilized broth.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light red-beige.

Prepared Appearance: Prepared medium without carbohydrates is bright red and clear.

Expected Cultural Response: Cultural response on Phenol Red Broth Base at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Proteus vulgaris</i> ATCC® 13315	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate tubes with isolated colonies.
2. An inverted Durham tube, added to the broth medium prior to sterilization, is used to detect gas production.
3. Incubate at $35 \pm 2^{\circ}\text{C}$ for 18 – 24 hours with loose caps.
4. Examine tubes for growth, acid production, and gas production (if Durham tube is used).

Results

A yellow color in the medium indicates a positive reaction for carbohydrate fermentation. If a Durham tube is used, bubbles in the inverted tube is an indication of gas production. The presence of a single bubble is recorded as positive for the production of gas.⁷

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. The addition of some carbohydrates to the basal medium may result in an acid reaction. To ensure accuracy of interpretation, uninoculated control tubes and/or inoculated Phenol Red Broth Base control tubes should be run in parallel with the fermentation tests.

Packaging

Phenol Red Broth Base	Code No.	7148A	500 g
		7148B	2 kg
		7148C	10 kg

References

1. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Vera, H. D.** 1950. Relation of peptones and other culture media ingredients to accuracy of fermentation tests. Am. J. Public Health. **40**:1267.
4. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, MD.
5. **Vanderzant, C., and D. F. Splittstoesser.** 1992. Compendium of methods for the microbiological examination of food. American Public Health Association, Washington, D.C.
6. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International. AOAC International, Arlington, VA.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PHENYLETHANOL AGAR (7147)

Intended Use

Phenylethanol Agar is used with blood for the selective isolation of gram-positive cocci.

Product Summary and Explanation

Brewer and Lilley^{1,2} reported the addition of phenylethanol to a nutritive medium permitted growth of gram-positive organisms, but markedly to completely inhibited growth of gram-negative organisms. Phenylethanol Agar inhibits swarming of *Proteus* spp., and can be used to selectively isolate anaerobic bacteria from clinical specimens with mixed flora. Phenylethanol Agar is specified for use in several reference methods.^{3,4}

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal. Sodium Chloride maintains the osmotic balance of the medium. Phenylethanol is bacteriostatic for gram-negative bacteria and inhibits DNA synthesis. Agar is the solidifying agent. The addition of 5% defibrinated sheep blood to the basal medium can enhance microorganism recovery of the medium.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Phenylethanol	2.5 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Irritating to eyes, respiratory system, and skin. May cause harm to unborn child.

Directions

1. Suspend 42.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, beige with soft lumps.

Prepared Appearance: Prepared medium is clear to slightly hazy and pale yellow. Prepared medium with 5% sheep blood is red and opaque.

Expected Cultural Response: Cultural response on Phenylethanol Agar at 35°C after 18 - 24 hours incubation in an aerobic atmosphere.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	markedly inhibited	---
<i>Enterococcus faecalis</i> ATCC® 29212	growth	beta hemolysis
<i>Proteus mirabilis</i> ATCC® 12453	markedly inhibited	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis
<i>Staphylococcus epidermidis</i> ATCC® 12228	growth	no hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto surface of the medium. Streak for isolation with inoculating loop.
2. Incubate plates at 35°C under conditions of increased CO₂ (5 - 10%) for 18 – 24 hour, and if necessary, 40 – 48 hours.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. Perform additional biochemical testing to identify the organism.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. *Pseudomonas aeruginosa* is not completely on this medium.⁶
2. Some gram-positive cocci may be slightly inhibited and many require further incubation (up to 48 hours) for sufficient growth to be evident.⁷
3. Subculture gram-positive colonies onto nonselective medium for biochemical testing.⁷

Packaging

Phenylethanol Agar	Code No.	7147A	500 g
		7147B	2 kg
		7147C	10 kg

References

1. **Brewer, J. H., and B. D. Lilley.** 1949. Paper presented at the December meeting of the Maryland Association of Medical and Public Health Laboratories.
2. **Lilley, B. D., and J. H. Brewer.** 1953. The selective antibacterial action of phenylethylalcohol. J. Pharm. Assoc. **42**:6.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
4. **Isenberg, H. D.** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
5. **Washington, J. A., Jr.** 1981. Laboratory procedures in clinical microbiology. Springer-Verlag, New York.
6. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
7. **MacFaddin, J. F.** 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol. 1 Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PHOSPHATE BUFFER, pH 7.2 (7380)

Intended Use

Phosphate Buffer, pH 7.2 is used for the preparation of microbiological dilution blanks.

Product Summary and Explanation

The formula for phosphate buffer is specified by the American Public Health Association (APHA) for use in diluting test samples. Phosphate Buffer, pH 7.2 is specified for use in diluting water, dairy products and food for microbiological methods. In the examination of water¹ and dairy products² the addition of magnesium chloride is recommended.

This buffer is also referred to as Butterfield's Buffered Phosphate Diluent and recommended for examination of food.² Phosphate Buffer, pH 7.2 stabilizes the pH of water used for dilutions.

Principles of the Procedure

Phosphate Buffer, pH 7.2 is used in the preparation of dilution blanks for use in microbiological testing. Phosphate Buffer is used rather than unbuffered water in order to standardize this potential variable due to the wide variation in the pH of distilled water from multiple sources.

Formula / Liter

Potassium Dihydrogen Phosphate..... 26.22 g

Sodium Carbonate 7.78 g

Final pH: 7.2 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Magnesium Chloride, 5 mL

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

Stock Solution

1. Dissolve 34 g of the medium in one liter of purified water until evenly dissolved.
2. Autoclave at 121°C for 15 minutes, if desired. Store under refrigeration.

Working Solution

1. Add 1.25 mL of Stock Solution and 5 mL of a magnesium chloride solution (81.1 g MgCl₂ • 6H₂O per liter of purified water) to purified water and make up to one liter.
2. Dispense into bottles or tubes to provide 99 ± 2.0 mL, 9 ± 0.2 mL or other appropriate quantities.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and white.

Prepared Appearance: Prepared buffer is clear and colorless.

Expected Cultural Response²: Cultural response of *Escherichia coli* on Violet Red Bile Agar (32°C, 18 - 24 hours) after holding for one hour at room temperature in the working solution.

Microorganism	Response (Toxicity Test)
<i>Escherichia coli</i> ATCC® 25922	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion and use of Phosphate Buffer, pH 7.2.

Results

Refer to appropriate references for results following test procedures.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Packaging

Phosphate Buffer, pH 7.2	Code No.	7380A	500 g
		7380B	2 kg
		7380C	10 kg

References

1. **Greenberg, Trussell, and Clesceri (eds.).** 1985. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington, D.C.
2. **Richardson. (ed.).** 1985. Standard methods for the examination of dairy products, 15th ed. American Public Health Association, Washington, D.C.
3. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

POTATO DEXTROSE AGAR (7149)

Intended Use

Potato Dextrose Agar is used for the cultivation of fungi.

Product 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 recommended for plate count methods for foods, dairy products¹⁻⁴ and testing cosmetics.³ It can be used for growing clinically significant yeast and molds.⁵ The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes.⁶

Principles of the Procedure

Potato Dextrose Agar is composed of dehydrated Potato Infusion and Dextrose that encourage luxuriant fungal growth. Agar is added as the solidifying agent. Many standard procedures use a specified amount of sterile tartaric acid (10%) to lower the pH of this medium to 3.5 ± 0.1 , inhibiting bacterial growth. Do not reheat the acidified medium, heating in the acid state will hydrolyze the agar.

Formula / Liter

Potato Infusion (dehydrated)..... 4 g
Dextrose..... 20 g
Agar 15 g

Final pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 39 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and pale to light yellow.

Expected Cultural Response: Cultural response on Potato Dextrose Agar at 25 - 35°C after 5 days of incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	good growth
<i>Candida albicans</i> ATCC® 10231	good growth
<i>Penicillium roquefortii</i> ATCC® 10110	good growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Pour Plate Methods^{1,3}

1. Add 1 mL of test sample to a sterile petri dish.
2. Add the specified amount (10 or 20 mL) of sterile, molten agar (cooled to 45 - 50°C) and swirl gently to mix well. Allow to solidify.
3. Incubate at 22 - 25°C or 30 - 32°C (depending on the method being followed) for 5 days or longer.

Results

Yeasts will grow as creamy to white colonies. Molds will grow as filamentous colonies of various colors. Count the number of colonies and consider the dilution factor (if the test sample was diluted) in determining the yeast and/or mold counts per gram or milliliter of material.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Potato Dextrose Agar	Code No.	7149A	500 g
		7149B	2 kg
		7149C	10 kg

References

1. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
2. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed., AOAC International, Gaithersburg, MD.
4. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
5. **Murray, P.R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Mac Faddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

POTATO DEXTROSE AGAR w/ LECITHIN & TWEEN 80 (7575)

Intended Use

Potato Dextrose Agar w/ Lecithin & Tween 80 is used for the isolation of fungi from surfaces sanitized with quaternary ammonium compounds.

Product 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. The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes.¹ Potato Dextrose Agar w/ Lecithin & Tween 80 is a modification of Potato Dextrose Agar. The addition of Lecithin and Tween 80 to Potato Dextrose Agar is used to neutralize antiseptics and disinfectants for environmental monitoring and other applications.² Complete neutralization of disinfectants is important. Disinfectant carryover can cause a false no-growth test result.

Principles of the Procedure

Potato Infusion provides nitrogen and vitamin sources required for organism growth. Dextrose is included as a carbon source. Lecithin neutralizes quaternary ammonium compounds and ethanol, and Tween 80 neutralizes phenols, hexachlorophene, and formalin. Agar is the solidifying agent.

Formula / Liter

Potato Infusion (dehydrated).....	4 g
Dextrose.....	20 g
Tween 80	5 g
Lecithin.....	0.7 g
Agar	15 g

Final pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 44.7 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, lumpy, and light yellow beige.

Prepared Appearance: Prepared medium is hazy and pale to light beige.

Expected Cultural Response: Cultural response on Potato Dextrose Agar w/ Lecithin & Tween 80 at 25 - 30°C after 2 - 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Bacillus subtilis</i> ATCC® 9372	partial inhibition
<i>Candida albicans</i> ATCC® 10231	partial inhibition
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	partial inhibition
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Salmonella typhimurium</i> ATCC® 19430	partial inhibition

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.

Results

Yeasts grow creamy to white colonies. Molds will grow as fuzzy colonies of various colors. Count the number of colonies and consider the dilution factor (if test sample was diluted) in determining the yeast and/or mold counts per gram or milliliter of material.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Potato Dextrose Agar w/ Lecithin & Tween 80	Code No.	7575A	500 g
		7575B	2 kg
		7575C	10 kg

References

1. **Sabouraud, R.** 1892. Ann. Dermatol. Syphilol. **3**:1061.
2. **Jarrett, L., and A. C. Sonnenwirth (eds.).** 1980. Gradwohl's and parasitic infections, 7th ed. American Public Health Association, Washington, D.C.
3. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (eds.).** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

POTATO DEXTROSE BROTH (7585)

Intended Use

Potato Dextrose Broth is used for the cultivation of fungi.

Product Summary and Explanation

Potato Dextrose Broth is a general purpose broth for yeasts and molds. The low pH of this medium inhibits bacterial growth. Potato Dextrose Broth is the same formula as Potato Dextrose Agar, but agar has been omitted.

Principles of the Procedure

Potato Dextrose Broth is composed of Potato Infusion Solids and Dextrose that encourage luxuriant fungal growth.

Formula / Liter

Potato Infusion Solids 4 g

Dextrose..... 20 g

Final pH: 5.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 24 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and yellow.

Expected Cultural Response: Cultural response in Potato Dextrose Broth at 25 - 35°C after 2 - 7 days of incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the isolation and identification of yeast and molds.

Results

Growth is indicated by turbidity.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Potato Dextrose Broth	Code No.	7585A	500 g
		7585B	2 kg
		7585C	10 kg

References

1. **Mac Faddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

POTATO INFUSION AGAR (7532)

Intended Use

Potato Infusion Agar is used for the isolation of *Brucella abortus*.

Product 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 Potato Infusion Agar 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 preparations of vaccines and antigens.

Principles of the Procedure

Infusion from Potatoes, Beef Extract, and Enzymatic Digest of Animal Tissue provides nitrogen, vitamins, and amino acids in Potato Infusion Agar. Dextrose and Glycerol are used as carbon sources. Sodium Chloride maintains the osmotic balance in the medium. Agar is the solidifying agent.

Formula / Liter

Potatoes, Infusion from Solids	200 g
Beef Extract	5 g
Enzymatic Digest of Animal Tissue.....	10 g
Dextrose.....	10 g
Sodium Chloride	5 g
Agar	15 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Glycerol, 20 mL

Precautions

1. For Laboratory Use.
2. *Brucella* spp. are classified as Biosafety Level 3 pathogens. All manipulations with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).¹

Directions

1. Suspend 49 g of the medium and 20 mL of glycerol in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light tan.

Prepared Appearance: Prepared medium is trace hazy with a slight precipitate and light amber.

Expected Cultural Response: Cultural response on Potato Infusion Agar at 35°C under 5 - 10% CO₂ for up to 72 hours of incubation.

Microorganism	Response
<i>Brucella abortus</i> ATCC® 4315	growth
<i>Brucella melitensis</i> ATCC® 4309	growth
<i>Brucella suis</i> ATCC® 4314	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Incubate plates at $35 \pm 2^{\circ}\text{C}$ in 5 - 10% CO_2 for 10 days.¹ Consult appropriate references for a complete discussion on the inoculation and identification of *Brucella* spp.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C . Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Best results are obtained on freshly prepared medium with a moist surface.

Packaging

Potato Infusion Agar	Code No.	7532A	500 g
		7532B	2 kg
		7532C	10 kg

References

1. **Moyer, N. P., and L. A. Holcomb.** 1995. *Brucella*, p. 549-555. In Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PRESENCE-ABSENCE BROTH (7500)

Intended Use

Presence-Absence Broth is used for the detection of coliform bacteria in water treatment plants or distribution systems using the presence-absence coliform test.

Product Summary and Explanation

The Presence-Absence (P-A) test is a presumptive detection for coliforms in water. The test is a simple modification of the multiple-tube procedure.¹ One 100 mL test sample is inoculated into a single culture bottle to obtain qualitative information on the presence or absence of coliforms, through 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.²⁻⁴

Comparative studies with the membrane filter procedure indicate 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 testing¹ and U.S. EPA.⁵

Principles of the Procedure

The nitrogen, vitamin, and amino acids sources are provided by Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Beef Extract. Lactose is the fermentable carbohydrate. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capacity. Sodium Chloride maintains the osmotic balance of the medium. 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 / Liter

Beef Extract	3 g
Enzymatic Digest of Gelatin	5 g
Lactose	7.46 g
Enzymatic Digest of Casein	9.83 g
Dipotassium Phosphate	1.35 g
Monopotassium Phosphate	1.35 g
Sodium Chloride	2.46 g
Sodium Lauryl Sulfate	0.05 g
Bromcresol Purple	0.0085 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Prepare triple strength concentration by adding 91.5 g of the medium in one liter of purified water.
2. Mix with frequent agitation to completely dissolve the medium and dispense 50 mL into a 250 mL screw-cap milk dilution bottle.
3. Autoclave at 121°C for 12 minutes. Cool and add 100 mL water sample.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear and red-purple to purple.

Expected Cultural Response: Cultural response in Presence-Absence Broth at 35°C after 18-48 hour incubation.

Microorganism	Response	Reactions (Color Change – Purple to Yellow)
<i>Enterococcus faecalis</i> ATCC® 29212	growth	negative
<i>Escherichia coli</i> ATCC® 25922	growth	positive
<i>Escherichia coli</i> ATCC® 11775	growth	positive
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate 50 mL of the sterile triple strength P-A Broth with 100 mL of the water sample.
2. Invert the bottle a few times to achieve an even distribution of the medium throughout the test sample. Incubate at 35 ± 0.5°C.
3. Inspect for acid and gas production after 24 and 48 hours incubation.

Results

An acid reaction from lactose fermentation is indicated by a distinct yellow color in the medium. Gas production is indicated by bubbles or foam present in the medium. 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.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. The P-A test is only a presumptive test for coliforms.
2. Confirmation and differentiation of coliforms detected by the P-A test may be achieved through biochemical testing, incubation time, and temperatures as outlined in appropriate references.^{1,5}
3. Extending 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.

Packaging

Presence-Absence Broth	Code No.	7500A	500 g
		7500B	2 kg
		7500C	10 kg

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Clark, J. A., and J. E. Pagel.** 1977. Pollution indicator bacteria associated with municipal raw and drinking water supplies. Can. J. Microbiol. **23**:465-470.
3. **Clark, J. A.** 1980. The influence of increasing numbers of nonindicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence tests. Can. J. Microbiol. **26**:827-832.
4. **Clark, J. A., C. A. Burger, and L. E. Sabatinos.** 1982. Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. Can. J. Microbiol. **28**:1002-1013.
5. **Federal Register.** 1989. National primary drinking water regulations; total coliforms (including fecal coliforms and *E. coli*). Fed Regist. **54**:27544-27568.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PSEUDOMONAS F AGAR (7312)

Intended Use

Pseudomonas F Agar is used for the detection and differentiation of *Pseudomonas aeruginosa* on the basis of fluorescein production.

Product Summary and Explanation

Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is the most frequently isolated nonfermentative bacillus in clinical specimens.¹ This organism is a significant cause of burn and nosocomial infections.² The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.¹

Pseudomonas aeruginosa produces a number of water-soluble pigments, including yellow-green or yellow-brown fluorescent pigment pyoverdin.² When pyoverdin combines with the blue water-soluble pigment pyocyanin, the bright green color characteristic of *Pseudomonas aeruginosa* is created.² Pseudomonas F Agar enhances the production of fluorescein by *Pseudomonas* and inhibits the formation of pyocyanin. Both pigments diffuse from *Pseudomonas* colonies into the medium when they grow. Fluorescein elaborated on Pseudomonas F Agar is a fluorescent yellow color.

Pseudomonas F Agar is patterned after the formulations described by King, Ward, and Raney,³ and modified to United States Pharmacopeia (USP) specifications.⁴

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, vitamins, and amino acids in Pseudomonas F Agar. Dipotassium Phosphate stimulates fluorescein production and has an inhibitory effect on pyocyanin, along with increasing the phosphorus content. Magnesium Sulfate provides necessary cations for the activation of fluorescein production. Agar is the solidifying agent. Glycerol is supplemented as a source of carbon.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Dipotassium Phosphate.....	1.5 g
Magnesium Sulfate	1.5 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Glycerol, 10 grams

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 38 g of the medium and 10 grams of glycerol in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slight hazy and yellow-beige.

Expected Cultural Response: Cultural response on Pseudomonas F Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Pseudomonas aeruginosa</i> ATCC® 10145	growth	green-yellow colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	green-yellow colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Obtain inoculum from a pure 18 – 24 hour culture of organism to be tested.
2. Inoculate plates or agar slants by streaking the surface.
3. Incubate at $35 \pm 2^{\circ}\text{C}$ for 18 – 24 hours.

Results

Examine colonies under ultraviolet light (Wood's lamp).⁵ Take care when using UV illumination because it may have a bactericidal effect. Be sure there is good growth before placing the culture under UV light.

A positive result is indicated by a light, bright green-yellow color diffusing into the agar with a fluorescent zone surrounding growth.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. The formation of nonpigmented colonies does not completely rule out *Pseudomonas aeruginosa*.
3. Further tests are necessary for confirmation of *Ps. aeruginosa*.

Packaging

Pseudomonas F Agar	Code No.	7312A	500 g
		7312B	2 kg
		7312C	10 kg

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
2. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
3. **King, E. O., M. K. Ward, and E. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301.
4. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PSEUDOMONAS ISOLATION AGAR (7329)

Intended Use

Pseudomonas Isolation Agar is used for the isolation of *Pseudomonas aeruginosa* and other *Pseudomonas* spp.

Product Summary and Explanation

Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is the most frequently isolated nonfermentative bacillus in clinical specimens.¹ This organism is a significant cause of burn and nosocomial infections.² The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.¹

Pseudomonas Isolation Agar is based on Medium A described by King, Ward, and Raney.³ This medium is especially useful for isolating *Pseudomonas* spp. from clinical specimens such as stools, wounds, and urine.¹ *Pseudomonas Isolation Agar* includes Irgasan®, a potent broad spectrum antimicrobial that is not active against *Pseudomonas* spp.⁴ *Pseudomonas Isolation Agar* is selective and formulated to enhanced formation of blue or blue-green pyocyanin pigment by *Pseudomonas aeruginosa*. The pigment diffuses into the medium surrounding growth.

Principles of the Procedure

Enzymatic Digest of Gelatin provides nitrogen, vitamins, and carbon in *Pseudomonas Isolation Agar*. 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. Glycerol serves as an energy source. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	20 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10 g
Irgasan®	0.025 g
Agar	13.6 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement /Liter

Glycerol, 20 mL

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 45 g of the medium in one liter of purified water containing 20 mL of glycerol.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to trace hazy and yellow-tan.

Expected Cultural Response: Cultural response on *Pseudomonas Isolation Agar* at 25°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Proteus mirabilis</i> ATCC® 12453	inhibited	---
<i>Pseudomonas aeruginosa</i> ATCC® 10145	growth	green to blue-green colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	green to blue-green colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate medium using the streak plate method to obtain isolated colonies.
2. Incubate for 18 – 48 hours at 35°C.

Results

Examine for presence of good growth. *Pseudomonas aeruginosa* colonies will be green to blue-green with pigment that diffuses into the medium.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.⁵
3. Non-*Pseudomonas aeruginosa* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Pseudomonas aeruginosa*.

Packaging

Pseudomonas Isolation Agar	Code No.	7329A	500 g
		7329B	2 kg
		7329C	10 kg

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
2. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
3. **King, E. O., M. K. Ward, and E. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301-307.
4. **Furia and Schenkel.** 1968. Soap and chemical specialties. January.
5. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.0-1.20.47. In H. D. Isenberg (ed.). Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PSEUDOMONAS ISOLATION BROTH (7474)

Intended Use

Pseudomonas Isolation Broth is used for the detection of *Pseudomonas* spp.

Product Summary and Explanation

Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is the most frequently isolated nonfermentative bacillus in clinical specimens.¹ This organism is a significant cause of burn and nosocomial infections.² The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.¹

Pseudomonas Isolation Broth is based on Medium A as described by King, Ward, and Raney.³ Pseudomonas Isolation Broth includes Irgasan®, a potent broad spectrum antimicrobial that is not active against *Pseudomonas* spp.⁴

Principles of the Procedure

Enzymatic Digest of Gelatin and Enzymatic Digest of Casein provides nitrogen, vitamins, and carbon in Pseudomonas Isolation Broth. 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. Glycerol serves as an energy source.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Enzymatic Digest of Casein	10 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10 g
Irgasan®	0.025 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement /Liter

Glycerol, 20 mL

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Dissolve 31.5 g of the medium and 20 mL of glycerol in 980 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to trace hazy and yellow-tan.

Expected Cultural Response: Cultural response in Pseudomonas Isolation Broth at 25°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Pseudomonas aeruginosa</i> ATCC® 10145	growth	blue-green
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	green
<i>Pseudomonas aeruginosa</i> ATCC® 35422	growth	pale green
<i>Proteus mirabilis</i> ATCC® 12453	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for the isolation and identification of *Pseudomonas* spp.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Pseudomonas Isolation Broth	Code No.	7474A	500 g
		7474B	2 kg
		7474C	10 kg

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
2. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
3. **King, E. O., M. K. Ward, and E. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301-307.
4. **Furia and Schenkel.** 1968. Soap and chemical specialties. January.

Technical Information

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PSEUDOMONAS P AGAR (7328)

Intended Use

Pseudomonas P Agar is used for the detection and differentiation of *Pseudomonas aeruginosa* on the basis of pyocyanin production.

Product Summary and Explanation

Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is the most frequently isolated nonfermentative bacillus in clinical specimens.¹ This organism is a significant cause of burn and nosocomial infections.² The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.¹

Pseudomonas aeruginosa produces a number of water-soluble pigments, including yellow-green or yellow-brown fluorescent pigment pyoverdin.² When pyoverdin combines with the blue water-soluble pigment pyocyanin, the bright green color characteristic of *Pseudomonas aeruginosa* is created.² Pseudomonas P Agar enhances the production of pyocyanin and inhibits the formation of fluorescein. Both pigments diffuse from *Pseudomonas* colonies into the medium when they grow. Pyocyanin elaborated on Pseudomonas P Agar is a blue color.

Pseudomonas P Agar is patterned after the formulations described by King, Ward, and Raney,³ and modified to United States Pharmacopeia (USP) specifications.⁴

Principles of the Procedure

Enzymatic Digest of Gelatin provides nitrogen, vitamins, and amino acids in Pseudomonas P Agar. Potassium Sulfate and Magnesium Chloride stimulate pyocyanin production. Agar is the solidifying agent. Glycerol is supplemented as a source of carbon.

Formula / Liter

Enzymatic Digest of Gelatin	20 g
Potassium Sulfate	10 g
Magnesium Chloride	1.5 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Glycerol, 10 g

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 46.4 g of the medium in one liter of purified water containing 10 g of glycerol.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to trace hazy and yellow-beige.

Expected Cultural Response: Cultural response on Pseudomonas P Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction
<i>Escherichia coli</i> ATCC® 25922	growth	--
<i>Pseudomonas aeruginosa</i> ATCC® 10145	growth	blue colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	blue-green colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Obtain inoculum from a pure 18 – 24 hour culture of organism to be tested.
2. Inoculate plates or agar slants by streaking the surface.
3. Incubate at 35 ± 2°C for 18 – 24 hours.

Results

Examine colonies for the presence of a blue, diffusable pigment.

A positive result is indicated by a blue pigment diffusing into the agar.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. The formation of nonpigmented colonies does not completely rule out *Pseudomonas aeruginosa*.
3. Further tests are necessary for confirmation of *Ps. aeruginosa*.

Packaging

Pseudomonas P Agar	Code No.	7328A	500 g
		7328B	2 kg
		7328C	10 kg

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
2. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
3. **King, E. O., M. K. Ward, and E. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301.
4. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

PURPLE LACTOSE AGAR (7251)

Intended Use

Purple Lactose Agar is used for the detection of coliform bacteria.

Product Summary and Explanation

Purple Lactose Agar is a modification of Litmus Lactose Agar, described by Wurtz.¹ In Purple Lactose Agar, bromcresol purple replaces litmus, which is less selective and less stable. Purple Lactose Agar is used for detecting coliforms, and for differential studies based on fermentation of lactose. Colonies of lactose-fermenting organisms are differentiated from lactose non-fermenters by color change of the indicator from blue-purple (alkaline) to yellow (acid).² If gas is produced during fermentation of the carbohydrate, bubbles will appear in the medium.²

Principles of the Procedure

Beef Extract and Peptone provide carbon, nitrogen, and amino acids used for general growth requirements in Purple Lactose Agar. Lactose is the carbohydrate used in the fermentation reaction. Bromcresol Purple is the pH indicator. Agar is the solidifying agent.

Formula / Liter

Beef Extract 3 g
Peptone..... 5 g
Lactose 10 g
Bromcresol Purple 0.025 g
Agar 10 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 28 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and grey-purple.

Prepared Appearance: Prepared medium is trace to slightly hazy and purple.

Expected Cultural Response: Cultural response on Purple Lactose Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions	
		Acid	Gas
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	positive	positive
<i>Escherichia coli</i> ATCC® 25922	growth	positive	positive
<i>Salmonella typhi</i> ATCC® 19430	growth	negative	negative
<i>Staphylococcus aureus</i> ATCC® 25923	growth	positive	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on expected reactions of *Enterobacteriaceae* spp.^{2,3}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Purple Lactose Agar	Code No.	7251A	500 g
		7251B	2 kg
		7251C	10 kg

References

1. **Wurtz.** 1897. *Technique Bacteriologique*. Masson, Paris.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Isenberg, H. D. (ed.).** 1992. *Clinical microbiology procedures handbook*, vol. 1. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

R2A AGAR (7390)

Intended Use

R2A Agar is used for the enumeration and cultivation of bacteria from potable water.

Product Summary and Explanation

R2A Agar was developed by Reasoner and Geldreich¹ for bacterial plate counts of treated potable water. R2A Agar is a low nutrient medium, and in combination with a lower incubation temperature and longer incubation time, stimulates the growth of stressed and chlorine-tolerant bacteria.¹ Nutritionally rich media support the growth of fast-growing bacteria, and may suppress slow growing or stressed bacteria found in treated water. When compared with Tryptone Glucose Yeast Extract Agar or Standard Methods Agar, R2A Agar reported improved recovery of stress and chlorine-tolerant bacteria from drinking water systems.^{2,3,4}

R2A Agar is recommended in standard methods for pour plate, spread plate, and membrane filter methods for heterotrophic plate counts.⁵

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Acid Hydrolysate of Casein provide nitrogen, carbon and minerals in R2A Agar. Yeast Extract is a source of vitamins and trace elements. Dextrose serves as a carbon source in the formula. Soluble Starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Dipotassium Phosphate is used to balance the pH, and Magnesium Sulfate Heptahydrate is a source of divalent cations and sulfate. Sodium Pyruvate increases the recovery of stressed cells. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	0.25 g
Enzymatic Digest of Animal Tissue.....	0.25 g
Acid Hydrolysate of Casein	0.5 g
Yeast Extract.....	0.5 g
Dextrose.....	0.5 g
Soluble Starch.....	0.5 g
Dipotassium Phosphate.....	0.3 g
Magnesium Sulfate Heptahydrate.....	0.1 g
Sodium Pyruvate.....	0.3 g
Agar	15 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 18.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and light beige.

Expected Cultural Response: Cultural response in R2A Agar at 35°C after 1 - 5 days incubation.

Microorganism	Response
<i>Enterococcus faecalis</i> ATCC® 29212	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Prepare test dilutions for heterotrophic plate count.
2. 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.
3. 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

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.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. R2A Agar is intended for use only with treated potable water.
3. 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.^{6,7}
4. Incubation time longer than indicated above may be necessary to recover additional slow-growing bacteria.

Packaging

R2A Agar	Code No.	7390A	500 g
		7390B	2 kg
		7390C	10 kg

References

1. **Reasoner, D. J., and E. E. Geldreich.** 1979. A new medium for the enumeration and subculture of bacteria from potable water. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.
2. **Fiksdal, L., E. A. Vik, A. Mills, and T. Staley.** 1982. Non-standard methods for enumerating bacteria in drinking water. Journal AWWA. **74**:313-318.
3. **Kelly, A. J., C. A. Justice, and L. A. Nagy.** 1983. Predominance of chlorine tolerant bacteria in drinking water systems. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. Q122.
4. **Means, E. G., L. Hanami, H. F. Ridgway, and B. H. Olson.** 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. Journal AWWA. **53**:585-590.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **VanSoestberger, A. A., and C. H. Lee.** 1969. Pour plates or streak plates? Appl. Microbiol. **18**:1092.
7. **Klein, D. A., and S. Wu.** 1974. Stress: a factor to be considered in heterotrophic microorganisms enumeration from aquatic environments. Appl. Microbiol. **27**:429.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

RAPPAPORT-VASSILIADIS (MSRV) MEDIUM SEMISOLID MODIFIED (7511)

Intended Use

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modified is used with novobiocin for the rapid detection of motile *Salmonella* spp.

Product Summary and Explanation

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modified is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella* spp. in feces and food products.^{1,2} The original research on MSRV Medium revealed a semisolid could be used as a rapid and sensitive test for isolating motile *Salmonella* from food products following pre-enrichment or selective enrichment.^{3,4} The semisolid medium allows motility to be detected as halos of growth around the original point of inoculation.

MSRV Medium is recommended by the European Chocolate Manufacturer's Association. A collaborative study performed with support of American Cocoa Research Institute and the Canadian Chocolate Manufacturer's Association resulted in first action adoption of the MSRV method by AOAC International.⁵ MSRV Medium may be used as a plating medium for isolating *Salmonella* spp. (other than *S. typhi* and *S. paratyphi* type A) from stool specimens with high sensitivity and specificity.⁶

Principles of the Procedure

Enzymatic Digest of Casein and Casein Acid Hydrolysate are carbon and nitrogen sources used for general growth requirements in MSRV Medium. Sodium Chloride maintains the osmotic balance in the medium. Magnesium Chloride raises the osmotic pressure, and Potassium Dihydrogen Phosphate acts as a buffer. Malachite Green Oxalate is inhibitory to organisms other than *Salmonella* spp. Novobiocin is added as a selective agent. The low pH of the medium, combined with the presence of Malachite Green Oxalate, Magnesium Chloride, and Novobiocin, select for the highly resistant *Salmonella* spp. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	4.59 g
Casein Acid Hydrolysate	4.59 g
Sodium Chloride	7.34 g
Potassium Dihydrogen Phosphate.....	1.47 g
Magnesium Chloride, Anhydrous.....	10.93 g
Malachite Green Oxalate	0.037 g
Agar	2.7 g

Final pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

2% Novobiocin, 1 mL

Precautions

1. For In vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 31.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. **DO NOT AUTOCLAVE.**
4. Cool medium to 45 - 50°C and aseptically add 1 mL of a 2% filtered sterilized aqueous solution of novobiocin.
5. Mix well and dispense into petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light blue.

Prepared Appearance: Prepared medium is trace to slightly hazy and turquoise blue.

Expected Cultural Response: Cultural response inoculated with three drops (approximately 0.1 mL) of a 1/1000 dilution from an overnight broth culture onto MSRV Medium, incubated at 42°C for 18 – 24 hours.

Microorganism	Response	Reactions
<i>Citrobacter freundii</i> ATCC® 8090	partial to complete inhibition	---
<i>Pseudomonas aeruginosa</i> ATCC® 27853	inhibited	---
<i>Salmonella choleraesuis</i> ATCC® 13076	growth	motility halo
<i>Salmonella typhimurium</i> ATCC® 14028	growth	motility halo

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure^{5,7}

Pre-Enrichment: 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.⁷ Incubate at 35°C for 20 ± 2 hours.

Selective Enrichment: Inoculate 10 mL of Tetrathionate Broth (prewarmed to 35°C) with 1 mL of the pre-enrichment culture. Incubate at 35°C for 8 ± 0.5 hours.

Motility Enrichment on MSRV: After selective enrichment incubation, mix the broth culture. Inoculate 3 drops at separate spots on an MSRV plate. Incubate at 42 ± 0.5°C for 16 ± 0.5 hours.

Results

Positive: Growth of migrated cells is visible as a grey-white, turbid zone extending out from the inoculated drop. Test sample is considered presumptively positive for motile *Salmonella* spp.

Negative: Medium remains blue-green around inoculation drops, with no grey-white, turbid zone extending out from the drop. Test sample is considered negative for motile *Salmonella* spp.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

The combined inhibitory factors of this medium may inhibit certain *Salmonella*, such as *S. typhi* and *S. choleraesuis*. Isolation techniques should include a variety of enrichment broths and isolation media.

Packaging

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modified	Code No.	7511A	500 g
		7511B	2 kg
		7511C	10 kg

References

1. Rappaport, F., N. Konforti, and B. Navon. 1956. A new enrichment medium for certain salmonellae. J. Clin. Pathol. **9**:261-266.
2. Vassiliadis, P., D. Trichopoulos, A. Kalandidi, and E. Xirouchaki. 1978. Isolation of salmonellae from sewage with a new procedure of enrichment. J. Appl. Bacteriol. **44**:233-239.
3. DeSmedt, J. M., R. Bolderdijk, H. Rappold, and D. Lautenschlaeger. 1986. Rapid *Salmonella* detection in food by motility enrichment on a modified semi-solid Rappaport-Vassiliadis Medium. J. Food Prot. **49**:510-514.
4. DeSmedt, J. M., and R. Bolderdijk. 1987. Dynamics of *Salmonella* isolation with modified semi-solid Rappaport-Vassiliadis Medium. J. Food Prot. **50**:658-661.
5. DeSmedt, J. M., R. Bolderdijk, and J. Milas. 1994. *Salmonella* detection in cocoa and chocolate by motility enrichment on modified semi-solid Rappaport-Vassiliadis Medium: a collaborative study. J. AOAC Int. **77**:365-373.
6. Dusch, H., and M. Altwegg. 1995. Evaluation of five new plating media for isolation of *Salmonella* spp. J. Clin. Micro. **33**:802-804.
7. Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana. 1995. *Salmonella*. p. 5.01-5.20. In FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. at TEL (800)783-3213 in the US/Canada or (410)780-5120 and FAX (800)875-8563 in the US/Canada or (410)780-5470 for Technical Service on questions involving dehydrated culture media preparation or performance.

RAPPAPORT-VASSILIADIS R10 BROTH (7512)

Intended Use

Rappaport-Vassiliadis R10 Broth is used for the selective enrichment of *Salmonella* spp. from foods.

Product Summary and Explanation

Rappaport et al.¹ formulated an enrichment medium for *Salmonella* spp. that was modified by Vassiliadis et al.² The Rappaport formulation, designated R 25/37°C, recommended incubation at 37°C. The Vassiliadis modification, designated R 10/43°C, had a reduced level of Malachite Green and recommended incubation at 43°C. Peterz later showed that incubation at $41.5 \pm 0.5^\circ\text{C}$ 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. It has gained approval for use in analyzing milk and milk products,⁴ raw flesh foods, highly contaminated foods, and animal feeds.^{5,6} This medium selectively enriches for *Salmonella* spp. because bacteria, including other intestinal bacteria, are typically susceptible to or inhibited by Malachite Green, high osmotic pressure and/or low pH. *S. typhi* and *S. choleraesuis* are sensitive to Malachite Green and may be inhibited.

Principles of the Procedure

Enzymatic Digest of Casein is the carbon and nitrogen sources for general growth requirements in Rappaport-Vassiliadis R10 Broth. Magnesium Chloride raises the osmotic pressure in the medium, and Potassium Dihydrogen Phosphate acts as a buffer. Malachite Green Oxalate is inhibitory to organisms other than *Salmonella* spp. The low pH of the medium, combined with the presence of Malachite Green Oxalate and Magnesium Chloride, select for the highly resistant *Salmonella* spp.

Formula / Liter

Enzymatic Digest of Casein	4.54 g
Sodium Chloride	7.20 g
Potassium Dihydrogen Phosphate.....	1.45 g
Magnesium Chloride, Anhydrous.....	13.4 g
Malachite Green Oxalate	0.036 g
Final pH: 5.1 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 26.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 116°C (10 lb pressure) for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pale green.

Prepared Appearance: Prepared medium is dark green.

Expected Cultural Response: Cultural response in 10 mL of prepared Rappaport-Vassiliadis R10 Broth inoculated with 100 CFU and incubated at 41.5°C for 24 hours. After incubation, subculture to Brilliant Green Agar and incubate at 35°C for 18 - 24 hours incubation

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Proteus vulgaris</i> ATCC® 13315	inhibited
<i>Salmonella arizonae</i> ATCC® 13314	good growth
<i>Salmonella typhimurium</i> ATCC® 14028	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

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. 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 : Using aseptic technique, transfer the cottonwool plug or the pad to 100 mL of a suitable pre-enrichment medium such as Buffered Peptone Water. Incubate at 37°C for 18 - 24 hours.

Selective Enrichment: 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. Incubate Rappaport-Vassiliadis R10 Broth at $41.5 \pm 0.5^\circ\text{C}$. Incubate Muller Kauffman Tetrathionate Broth at $42 \pm 1^\circ\text{C}$ for 48 hours.

Results: After incubation, subculture both selective enrichment broths to Brilliant Green Agar and XLD Agar. Incubate at 35°C for 18 - 24 hours. Examine for typical *Salmonella* colonies. Confirm identification of isolates through biochemical and serological testing.

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.^{5,6}

Pre-Enrichment: 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.^{4,5,6} Incubate at 35°C for 24 ± 2 hours^{5,6} or at 37°C for 16 - 20 hours,⁴ depending on the referenced procedure being followed.

Selective Enrichment: 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 1 mL of the pre-enrichment culture.^{4,5,6} Incubate Rappaport-Vassiliadis R10 Broth at $41.5 \pm 0.5^\circ\text{C}$ for 24 ± 2 hours. Incubate the other selective enrichment broths appropriately.

Results: After incubation, subculture Rappaport-Vassiliadis R10 Broth and the other selective enrichment broths to selective agar media and incubate at $35 \pm 2^\circ\text{C}$ for 24 ± 2 hours.^{4,5} Examine for typical *Salmonella* colonies. Confirm identification of isolates through biochemical and serologic tests.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

The combined inhibitory factors of this medium may inhibit certain *Salmonella*, such as *S. typhi* and *S. choleraesuis*. Isolation techniques should include a variety of enrichment broths and isolation media.

Packaging

Rappaport-Vassiliadis R10 Broth	Code No.	7512A	500 g
		7512B	2 kg
		7512C	10 kg

References

1. Rappaport, F., N. Konforti, and B. Navon. 1956. A new enrichment medium for certain salmonellae. J. Clin. Pathol. 9:261-266.
2. Vassiliadis, P., D. Trichopoulos, A. Kalandidi, and E. Xirouchaki. 1978. Isolation of salmonellae from sewage with a new procedure of enrichment. J. Appl. Bacteriol. 44:233-239.
3. Peterz, M., C. Wiberg, and P. Norberg. 1989. The effect of incubation temperature and magnesium chloride concentration on growth of *Salmonella* in homemade and commercially available dehydrated Rappaport-Vassiliadis broths. J. Appl. Bacteriol. 66:523-528.
4. International Dairy Federation. 1995. Milk and milk products: detection of *Salmonella*. IDF Standard 93B:1005. Brussels, Belgium.
5. Cunniff, P. (ed.). 1995. Official Methods of Analysis AOAC International, 16thed., AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SABOURAUD BHI AGAR (7235)

Intended Use

Sabouraud BHI Agar is used for the cultivation of fungi.

Product Summary and Explanation

Sabouraud formulated Sabouraud Dextrose Agar as a general purpose medium for the recovery of dermatophytes.¹ Brain Heart Infusion is a highly nutritious medium used for cultivating a variety of fastidious organisms and medically important fungi.² Sabouraud BHI Agar, developed by Gorman, combines ingredients of Sabouraud Dextrose Agar and Brain Heart Infusion.³ This medium is particularly useful for maximum recovery of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from body tissues and fluids, and as a primary recovery medium for saprophytic and pathogenic fungi.⁴

Selectivity can be obtained by adding chloramphenicol and cycloheximide.

Principles of the Procedure

Brain Heart Infusion is a source of carbon, protein, and nutrients in Sabouraud BHI Agar. Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogens, minerals, and vitamins required for organism growth. The high concentration of Dextrose is included as an energy source. Sodium Chloride maintains the osmotic balance of the medium. Disodium Phosphate provides buffering capacity. Agar is the solidifying agent. Chloramphenicol and Cycloheximide are broad spectrum antimicrobials, inhibiting a wide variety of gram-negative and gram-positive bacteria when added to this medium.

Formula / Liter

Brain Heart Infusion (dehydrated).....	10 g
Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	7 g
Dextrose.....	21 g
Sodium Chloride	2.5 g
Disodium Phosphate.....	1.25 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 59 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

OPTIONAL: To prepare selective medium, aseptically add a sterile solution containing chloramphenicol (0.05 g) and cycloheximide (0.5 g) per liter of sterile medium cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and medium to dark amber.

Expected Cultural Response: Cultural response on Sabouraud BHI Agar at 25 - 30°C after 2-7 days incubation (without the addition of antimicrobials).

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate Sabouraud BHI Agar tubes/plates with specimen.
2. Incubate media at 25-30°C for up to 7 days.

Results

Observe Sabouraud BHI Agar tubes or plates for growth and record colony morphology.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi. Non-selective fungal media should be used concurrently with selective media when isolating fungi.

Packaging

Sabouraud BHI Agar	Code No.	7235A	500 g
		7235B	2 kg
		7235C	10 kg

References

1. **Sabouraud, R.** 1892. Ann. Dermatol. Syphilol. **3**:1061.
2. **Dixon, D. M., and R. A. Fromtling.** 1995. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Gorman, J. W.** 1967. Sabhi, a new culture medium for pathogenic fungi. Am. J. Med. Technol. **33**:151.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SABOURAUD DEXTROSE AGAR (7150)

Intended Use

Sabouraud Dextrose Agar is used for the cultivation of fungi.

Product Summary and Explanation

Sabouraud Dextrose Agar (SDA) is a modification of Dextrose Agar described by Sabouraud.¹ SDA is used for cultivating pathogenic & commensal fungi and yeasts. The high dextrose concentration and acidic pH of the formula permits selectivity of fungi.² George³ enhanced SDA with the addition of cycloheximide, streptomycin, and penicillin to produce an excellent medium for the primary isolation of dermatophytes.

Sabouraud Dextrose Agar is used for determining the microbial content of cosmetics,⁴ in the mycological evaluation of food,^{5,6} and clinically to aid in the diagnosis of yeast and fungal infections.^{7,8}

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide the nitrogen and vitamin source required for organism growth in Sabouraud Dextrose Agar. The high concentration of Dextrose is included as an energy source. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein 5 g

Enzymatic Digest of Animal Tissue..... 5 g

Dextrose..... 40 g

Agar 15 g

Final pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 65 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and light beige.

Prepared Appearance: Prepared medium is clear to trace hazy and light amber.

Expected Cultural Response: Cultural response on Sabouraud Dextrose Agar at 25 - 30°C after 2 – 7 days of incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Microsporum canis</i> ATCC® 36299	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.

Results

Yeasts grow creamy to white colonies. Molds will grow as filamentous colonies of various colors. Count the number of colonies and consider the dilution factor (if the test sample was diluted) in determining the yeast and/or mold counts per gram or milliliter of material.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi.
3. Avoid overheating a medium with an acidic pH, this may result in a soft medium.

Packaging

Sabouraud Dextrose Agar	Code No.	7150A	500 g
		7150B	2 kg
		7150C	10 kg

References

1. **Sabouraud, R.** 1892. Ann. Dermatol. Syphilol. **3**:1061.
2. **Jarett, L., and A. C. Sonnenwirth (eds.).** 1980. Gradwohl's and parasitic infections, 7th ed. American Public Health Association, Washington, D.C.
3. **Georg, L. K., L. Ajello, and C. Papageorge.** 1954. Use of cycloheximide in the selective isolation of fungi pathogenic to man. J. Lab Clin. Med., **44**:422-428.
4. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (eds.).** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
5. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
7. **Murray, P.R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SABOURAUD DEXTROSE AGAR w/ CHLORAMPHENICOL (7306)

Intended Use

Sabouraud Dextrose Agar w/ Chloramphenicol is used for the selective isolation of fungi.

Product Summary and Explanation

Sabouraud Dextrose Agar (SDA) is a modification of Dextrose Agar described by Sabouraud.¹ SDA is used for cultivating pathogenic and commensal fungi and yeasts. The high dextrose concentration and acidic pH of the formula permits selectivity of fungi.² Sabouraud Dextrose Agar is used for determining the microbial content of cosmetics,³ in the mycological evaluation of food,^{4,5} and clinically to aid in the diagnosis of yeast and fungal infections.^{6,7}

Sabouraud Dextrose Agar w/ Chloramphenicol is a modification of SDA, with the addition of Chloramphenicol to increase selectivity.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide the nitrogen and vitamin sources required for organism growth in Sabouraud Dextrose Agar w/ Chloramphenicol. The high concentration of Dextrose is included as an energy source. Chloramphenicol is a broad-spectrum antibiotic inhibitory to a wide range of gram-negative and gram-positive bacteria. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Dextrose.....	40 g
Chloramphenicol.....	0.05 g
Agar	15 g

Final pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. VERY TOXIC. Toxic by inhalation and contact with skin.

Directions

1. Suspend 65 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and clear.

Expected Cultural Response: Cultural response on Sabouraud Dextrose Agar w/ Chloramphenicol at 25 - 30°C after 2 - 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Microsporum canis</i> ATCC® 36299	growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures on the isolation and identification of yeast and molds.

Results

Yeasts grow creamy to white colonies. Molds will grow as filamentous colonies of various colors. Refer to appropriate references for a complete discussion on yeast and molds.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi.

Packaging

Sabouraud Dextrose Agar w/ Chloramphenicol	Code No.	7306A	500 g
		7306B	2 kg
		7306C	10 kg

References

1. **Sabouraud, R.** 1892. Ann. Dermatol. Syphilol. 3:1061.
2. **Jarett, L., and A. C. Sonnenwirth (eds.).** 1980. Gradwohl's and parasitic infections, 7th ed. American Public Health Association, Washington, D.C.
3. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (eds.).** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed. AOAC International, Gaithersburg, MD.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, MD.

Technical Information

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SABOURAUD DEXTROSE AGAR w/ LECITHIN & TWEEN 80 (7392)

Intended Use

Sabouraud Dextrose Agar w/ Lecithin & Tween 80 is used for the isolation of fungi from surfaces sanitized with quaternary ammonium compounds.

Product Summary and Explanation

Sabouraud Dextrose Agar (SDA) is a modification of Dextrose Agar described by Sabouraud.¹ SDA is used for cultivating pathogenic and commensal fungi and yeasts. The high dextrose concentration and acidic pH of the formula permits selectivity of fungi.² The addition of Lecithin and Tween 80 to Sabouraud Dextrose Agar is used to neutralize antiseptics and disinfectants for environmental monitoring and other applications.³ Complete neutralization of disinfectants is important. Disinfectant carryover can cause false no-growth test results.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen and vitamin sources required for organism growth. Dextrose is included as an energy source. Lecithin neutralizes quaternary ammonium compounds and ethanol and Tween 80 neutralizes phenols, hexachlorophene and formalin. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Dextrose.....	40 g
Lecithin.....	0.7 g
Tween 80	5 g
Agar	15 g

Final pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 71 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.
4. After cooling to 45 - 50°C aseptically pour approximately 17 mL into 65 x 15 mm plates to give a meniscus of agar which extends above the top of the plate.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, lumpy, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and pale yellowish white in color.

Expected Cultural Response: Cultural response on Sabouraud Dextrose Agar w/ Lecithin & Tween 80 at 25 - 30°C after 2 - 7 days of incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures.

Results

Yeasts grow creamy to white colonies. Molds will grow fuzzy colonies of various colors. Count the number of colonies and consider the dilution factor (if the test sample was diluted) in determining the yeast and/or mold counts per gram or milliliter of material.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not homogeneous or appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Sabouraud Dextrose Agar w/ Lecithin & Tween 80	Code No.	7392A	500 g
		7392B	2 kg
		7392C	10 kg

References

1. **Sabouraud, R.** 1892. Ann. Dermatol. Syphilol. 3:1061.
2. **Jarrett, L., and A. C. Sonnenwirth (eds.).** 1980. Gradwohl's and parasitic infections, 7th ed. American Public Health Association, Washington, D.C.
3. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (eds.).** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SABOURAUD DEXTROSE AGAR, EMMONS (7204)

Intended Use

Sabouraud Dextrose Agar, Emmons is used for the cultivation of fungi.

Product Summary and Explanation

Sabouraud Dextrose Agar (SDA) is a modification of Dextrose Agar described by Sabouraud.¹ SDA is used for cultivating pathogenic and commensal fungi and yeasts. Emmons modified the formula by adjusting the pH and reducing the dextrose concentration.² This neutral pH enhances recovery of some pathogenic fungi. Sabouraud Dextrose Agar, Emmons is an excellent basal medium, and antibiotics and inhibitors may be added for the selective cultivation and isolation of various microorganisms.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide the nitrogen and vitamin sources required for organism growth in Sabouraud Dextrose Agar, Emmons. Dextrose is included as an energy source. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein 5 g
Enzymatic Digest of Animal Tissue..... 5 g
Dextrose..... 20 g
Agar 17 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 47 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to trace hazy and light amber in color.

Expected Cultural Response: Cultural response on Sabouraud Dextrose Agar, Emmons at 25 - 30°C after 2 – 7 days of incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Microsporum canis</i> ATCC® 36299	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Consult appropriate references for recommended test procedures using Sabouraud Dextrose Agar, Emmons.

Results

Yeasts grow creamy to white colonies. Molds will grow as fuzzy colonies of various colors. Count the number of colonies and consider the dilution factor (if test sample was diluted) to determine the yeast and/or mold counts per gram or milliliter of material.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi.

Packaging

Sabouraud Dextrose Agar, Emmons	Code No.	7204A	500 g
		7204B	2 kg
		7204C	10 kg

References

1. **Sabouraud, R.** 1892. Ann. Dermatol. Syphilol. 3:1061.
2. **Emmons, C. W., C. H. Binford and J. P. Uty.** 1970. Medical mycology, 2nd ed., Philadelphia: Lea and Febiger.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SALMONELLA SHIGELLA AGAR (7152)

Intended Use

Salmonella Shigella Agar is used for the isolation of *Salmonella* spp. and some strains of *Shigella* spp.

Product Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes a mild, self-limiting illness. Typhoid fever, caused by *Salmonella typhi*, is characterized by fever, headache, diarrhea, abdominal pain, and can result in fatal respiratory, hepatic, and or neurological damage.¹ This infection can result from the consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella* spp.

Shigellosis, caused by *Shigella* spp., is an intestinal illness characterized by abdominal pain, fever, and watery diarrhea. When associated with outbreaks, shigellosis is usually transmitted through contaminated food and/or water.

Salmonella Shigella Agar is a modification of the Desoxycholate Citrate Agar described by Leifson.² Salmonella Shigella Agar is superior to a number of other media for the isolation of *Salmonella* spp. and *Shigella* spp.³ Salmonella Shigella Agar is recommended for testing clinical specimens and food testing for the presence of *Salmonella* spp. and some *Shigella* spp.^{1,4,5}

Principles of the Procedure

Beef Extract, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue provide sources of nitrogen, carbon, and vitamins required for organism growth. Lactose is the carbohydrate present in Salmonella Shigella Agar. Bile Salts, Sodium Citrate and Brilliant Green inhibit gram-positive bacteria, most coliform bacteria, and inhibit swarming *Proteus* spp., while allowing *Salmonella* spp. to grow. Sodium Thiosulfate and Ferric Citrate permit detection of hydrogen sulfide by the production of colonies with black centers. Neutral Red is the pH indicator.

Formula / Liter

Beef Extract	5 g
Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Lactose	10 g
Bile Salts	8.5 g
Sodium Citrate	8.5 g
Sodium Thiosulfate	8.5 g
Ferric Citrate	1 g
Brilliant Green	0.00033 g
Neutral Red.....	0.025 g
Agar	13.5 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful if swallowed or inhaled. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 60 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light to medium pinkish-beige.

Prepared Appearance: Prepared medium is light to medium reddish-orange to peach, trace to slightly hazy.

Expected Cultural Response: Cultural response at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	complete inhibition	---
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	pink to rose-red colonies with precipitate
<i>Salmonella typhimurium</i> ATCC® 14028	growth	colorless colonies with black centers
<i>Shigella flexneri</i> ATCC® 12022	growth	colorless colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For isolation of *Salmonella* spp. and *Shigella* spp. from clinical specimens, inoculate fecal samples and rectal swabs onto one quadrant of Salmonella Shigella Agar, streak for isolation. Incubate plates at 35°C, and examine after 24 and 48 hours for colonies resembling *Salmonella* spp. or *Shigella* spp. Consult appropriate references for food testing.

Results

Enteric organisms are differentiated by their ability to ferment lactose. *Salmonella* spp. and *Shigella* spp. are non-lactose fermenters and form colorless colonies on Salmonella Shigella Agar. H₂S positive *Salmonella* spp. produce black-center colonies. Some *Shigella* spp. are inhibited on Salmonella Shigella Agar. *E. coli* produces pink to red colonies and may have some bile precipitation.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Salmonella Shigella Agar is highly selective and not recommended as the primary isolation of *Shigella*.^{1,2,6} Some *Shigella* spp. may be inhibited.
2. A few nonpathogenic organisms may grow on Salmonella Shigella Agar. These organisms can be differentiated by their ability to ferment lactose and other confirmatory tests.

Packaging

Salmonella Shigella Agar	Code No.	7152A	500 g
		7152B	2 kg
		7152C	10 kg

References

1. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol **40**:581.
3. **Rose, H. M., and M. H. Kolodny.** 1942. The use of SS (*Shigella-Salmonella*) Agar for the isolation of Flexner Dysentery bacilli from the feces. J. Lab. Clin. Med. **27**:1081-1083.
4. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **Taylor, W. I., and B. Harris.** 1965. Isolation of shigellae. II. Comparison of plating media and enrichment broths. Am. J. Clin. Pathol. **44**:476.
7. **McFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SCHAEDLER AGAR (7153)

Intended Use

Schaedler Agar is used for the cultivation of anaerobic microorganisms.

Product Summary and Explanation

Anaerobic bacteria cause a variety of human infections including endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.^{1,2} Survival of anaerobic bacteria is dependent on their sensitivity to oxygen, nutritional requirements, appropriate collection, culture medium, and incubation time and temperature.³ Schaedler Agar is suitable for standard procedures used in cultivating anaerobic bacteria.³⁻⁵

Schaedler Agar is prepared according to the formulation described by Schaedler, Dubos, and Costello,⁶ and modified by Mata, Carrillo, and Villatoro.⁷ Modifications include reduced dextrose to avoid interference with hemolytic reactions, reduced yeast extract to avoid darkening of the medium, and adjusted sodium chloride and nitrogen concentrations.

Principles of the Procedure

Tryptic Soy Broth, Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Yeast Extract provide vitamins, nitrogen, and amino acids in Schaedler Agar. Dextrose is a carbon source. Tris (hydroxymethyl) Aminomethane is used to buffer the medium. Hemin (X factor) stimulates organism growth. L-Cystine is a reducing agent. Agar is the solidifying agent.

Formula / Liter

Tryptic Soy Broth.....	10 g
Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Yeast Extract.....	5 g
Dextrose.....	5 g
Tris (hydroxymethyl) Aminomethane	3 g
Hemin.....	0.01 g
L-Cystine	0.4 g
Agar	13.5 g

Final pH: 7.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 41.9 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light tan.

Prepared Appearance: Prepared medium is light to medium yellow-beige and clear to trace hazy.

Expected Cultural Response: Cultural response on Schaedler Agar at 35°C after 48 - 72 hours incubation.

Microorganism	Response
<i>Bacteroides fragilis</i> ATCC® 25285	growth
<i>Clostridium perfringens</i> ATCC® 13124	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion of aerobic and anaerobic bacteria from clinical specimens, refer to appropriate procedures outlined in the references.³⁻⁵ Refer to standard methods for the examination of bacteria in food.^{8,9}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. When supplemented with 5% blood, beta-hemolytic streptococci may produce a hemolytic reaction similar to alpha hemolysis because of the high dextrose concentration in Schaedler Agar.

Packaging

Schaedler Agar	Code No.	7153A	500 g
		7153B	2 kg
		7153C	10 kg

References

1. **Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadmony (eds.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
2. **Smith, L. D. S.** 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Schaedler, R. W., R. Dubos, and R. Costello.** 1965. The development of the bacterial flora in the gastrointestinal tract of mice. J. Exp. Med. **122**:59.
7. **Mata, L. J., C. Carrillo, and E. Villatoro.** 1969. Fecal microflora in healthy persons in the preindustrial region. Appl. Microbiol. **17**:596.
8. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
9. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SCHAEDLER BROTH (7154)

Intended Use

Schaedler Broth is used for the cultivation of anaerobic microorganisms.

Product Summary and Explanation

Anaerobic bacteria cause a variety of human infections including endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.^{1,2} Survival of anaerobic bacteria is dependent on their sensitivity to oxygen, nutritional requirements, appropriate collection, culture medium, and incubation time and temperature.³ Schaedler Broth is suitable for standard procedures used in cultivating anaerobic bacteria.³⁻⁵

Schaedler Broth is prepared according to the formulation described by Schaedler, Dubos, and Costello,⁶ and modified by Mata, Carrillo, and Villatoro.⁷ Modifications include reduced dextrose to avoid interference with hemolytic reactions, reduced yeast extract to avoid darkening of the medium, and adjusted sodium chloride and nitrogen concentrations.

Stalons, Thornsberry, and Dowell evaluated nine broth media in varied carbon dioxide atmospheres for their ability to support growth of anaerobic bacteria.⁸ Schaedler Broth in an atmosphere of 5%CO₂, 10% hydrogen, and 85% nitrogen exhibited the fastest and highest growth response.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Soybean Meal, Enzymatic Digest of Animal Tissue, and Yeast Extract provide vitamins, nitrogen, and amino acids in Schaedler Broth. Dextrose is a carbon source. Sodium Chloride maintains the osmotic balance of the medium. Tris (hydroxymethyl) Aminomethane and Dipotassium Phosphate are used to buffer the medium. Hemin (X factor) stimulates organism growth. L-Cystine is a reducing agent.

Formula / Liter

Enzymatic Digest of Casein	5.6 g
Enzymatic Digest of Soybean Meal	1 g
Enzymatic Digest of Animal Tissue.....	5 g
Yeast Extract.....	5 g
Sodium Chloride	1.7 g
Dipotassium Phosphate.....	0.82 g
Dextrose.....	5.82 g
Tris (hydroxymethyl) Aminomethane	3 g
Hemin.....	0.01 g
L-Cystine	0.4 g

Final pH: 7.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Dissolve 28.4 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light tan.

Prepared Appearance: Prepared medium is light to medium yellow-beige and clear to trace hazy.

Expected Cultural Response: Cultural response in Schaedler Broth at 35°C after 18 - 72 hours incubation.

Microorganism	Response
<i>Bacteroides fragilis</i> ATCC® 25285	growth
<i>Clostridium perfringens</i> ATCC® 13124	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion of aerobic and anaerobic bacteria from clinical specimens, refer to appropriate procedures outlined in the references.³⁻⁵ Refer to standard methods for the examination of bacteria in food.^{9,10}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Schaedler Broth	Code No.	7154A	500 g
		7154B	2 kg
		7154C	10 kg

References

1. **Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadmony (eds.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
2. **Smith, L. D. S.** 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Schaedler, R. W., R. Dubos, and R. Costello.** 1965. The development of the bacterial flora in the gastrointestinal tract of mice. J. Exp. Med. **122**:59.
7. **Mata, L. J., C. Carrillo, and E. Villatoro.** 1969. Fecal microflora in healthy persons in the preindustrial region. Appl. Microbiol. **17**:596.
8. **Stalons, D. R., C. Thornsberry, and V. R. Dowell, Jr.** 1974. Effect of culture medium and carbon dioxide concentration on growth of anaerobic bacteria commonly encountered in clinical specimens. Appl. Microbiol. **27**:1098-1104.
9. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
10. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SELECTIVE STREP AGAR, MODIFIED # 2 (7405)

Intended Use

Selective Strep Agar, Modified # 2 is used with blood for the selective isolation of group A streptococci.

Product Summary and Explanation

Group A streptococcal infections are the most common cause of bacterial pharyngitis in children 5 to 10 years old.¹ Selective Streptococcus Agar was developed by Roantree et al., for the isolation of group A beta-hemolytic streptococci.² Further improvement was made by adding yeast nucleic acid and maltose, promoting increased colony size and enhanced clarity of hemolytic zones produced by group A streptococci.^{3,4} This modified formula also increased inhibition of *Staphylococcus* spp.

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Beef Extract. Maltose is added as an energy source, and along with Nucleic Acid promotes organism growth and enhanced hemolytic zone sizes produced by group A streptococci. Sodium Chloride maintains the osmotic balance of the medium. Neomycin Sulfate, Polymyxin B Sulfate, and other selective agents are used to inhibit *Staphylococcus* spp. Agar is the solidifying agent. In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β -hemolytic streptococci.⁵ Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.¹

Formula / Liter

Enzymatic Digest of Casein	10 g
Beef Extract	6.7 g
Maltose	0.25 g
Nucleic Acid	6 g
Sodium Chloride	5 g
Neomycin Sulfate	0.002 g
Polymyxin B Sulfate	0.024 g
Selective Agents	0.00289 g
Agar	15 g

Final pH: 7.3 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 43 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Do not overheat.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light beige.

Prepared Appearance: Prepared medium without blood is amber, and clear to slightly hazy. With 5% sheep blood the medium is cherry red and opaque.

Expected Cultural Response: Cultural response at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.¹
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are three types of hemolysis on blood agar media described as:⁶

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and no change in the medium.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
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.¹
3. Atmosphere of incubation is known to influence hemolytic reactions of beta-hemolytic streptococci.¹ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Packaging

Selective Strep Agar, Modified # 2	Code No.	7405A	500 g
		7405B	2 kg
		7405C	10 kg

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
2. **Roantree, R. J., L. A. Rantz, and E. J. Haines.** 1958. A medium containing nucleic acid, maltose, and antibiotics for the isolation of group A hemolytic streptococci. J. Lab. Clin. Med. **52**:496-500.
3. **Bernheimer, A. W., and M. Rodbart.** 1948. The effect of nucleic acids and of carbohydrates on the formation of streptolysin. J. Exper. Med. **88**:149-168.
4. **Schweiz Ztschr.** 1955. Allg. Path. **18**:278-287.
5. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
6. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SELENITE BROTH (7155)

Intended Use

Selenite Broth is used for the selective enrichment of *Salmonella* spp.

Product Summary and Explanation

Selenite Broth was originated by Leifson,¹ while observing good recovery of *Salmonella* spp. and reduced growth of fecal coliforms. Selenite Broth is used as a selective enrichment for the cultivation of *Salmonella* spp. that may be present in small numbers and competing with intestinal flora. *Salmonella* organisms are also injured in food-processing procedures, including exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers.² Although injured cells may not form colonies on selective media, they cause infection if ingested.³ *Salmonella* spp. cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.⁴

Selenite Broth conforms with the American Public Health Association (APHA),⁵ and is specified for clinical applications.^{4,6} Many modifications of Selenite Broth exist, including Selenite Cystine Broth, from the original formula described as Selenite F Broth by Leifson.¹

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in Selenite Broth. Lactose is the fermentable carbohydrate. Sodium Phosphate is the buffer. A rise in pH decreases selective activity of Selenite. The acid produced by lactose fermentation helps to maintain a neutral pH. Sodium Selenite inhibits the growth of gram-positive bacteria and many gram-negative bacteria.

Formula/Liter

Enzymatic Digest of Casein 2.5 g
Enzymatic Digest of Animal Tissue..... 2.5 g
Lactose 4 g
Sodium Phosphate..... 10 g
Sodium Selenite..... 4 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful by inhalation and if swallowed. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 23 g of the medium in one liter of purified water.
2. Heat to boiling. Avoid overheating.
3. **DO NOT AUTOCLAVE.**

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and off-white.

Prepared Appearance: Prepared medium is clear, very pale yellow to pale yellow with a slight precipitate.

Expected Cultural Response: Cultural response is exhibited on MacConkey Agar after 18 to 24 hours incubation at 35°C after enrichment in Selenite Broth.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 11775	inhibited
<i>Salmonella typhi</i> ATCC® 19430	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Shigella sonnei</i> ATCC® 25931	growth

The organisms listed are the minimum that should be performed for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of *Salmonella* spp., refer to appropriate references.

Results

Refer to references for the characteristic growth of *Salmonella* spp.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Selenite Broth	Code No.	7155A	500 g
		7155B	2 kg
		7155C	10 kg

References

1. **Leifson, E.** 1939. New selenite selective enrichment medium for the isolation of typhoid and paratyphoid bacilli. *Am. J. Hyg.* **24**:423-432.
2. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of salmonellae in foods. *J. Food Prot.* **44**:385-386.
3. **Sorrells, K. M., M. L. Speck, and J. A. Warren.** 1970. Pathogenicity of *Salmonella gallinarum* after metabolic injury by freezing. *Appl. Microbiol.* **19**:39-43.
4. **Murray, P. R. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Vanderzant, C., and D.F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **Isenberg, H. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Technical Information

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SELENITE CYSTINE BROTH (7283)

Intended Use

Selenite Cystine Broth is used for the selective enrichment of *Salmonella* spp.

Product Summary and Explanation

Selenite Cystine Broth is based upon Selenite Broth described by Leifson¹. The Food and Drug Administration proposed Selenite Cystine Broth, as an enrichment medium for detecting *Salmonella* in food materials.²

Selenite Cystine Broth is recommended by AOAC, USP, and APHA as a selective enrichment medium for *Salmonella* spp., while inhibiting the growth of other gram-negative bacilli.³⁻⁵

Principles of the Procedure

Pancreatic Digest of Casein and Peptic Digest of Animal Tissue are used as nitrogen and vitamin sources in Selenite Cystine Broth. Lactose is the carbohydrate and Disodium Phosphate is the buffer. Sodium Selenite is the selective agent against gram-positive bacteria and most enteric gram-negative bacilli. L-Cystine is a reducing agent.

Formula/Liter

Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Lactose	4 g
Sodium Phosphate.....	10 g
Sodium Selenite.....	4 g
L-Cystine.....	0.01 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful by inhalation and if swallowed. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 23 g of the medium in one liter of purified water.
2. Heat to boiling to completely dissolve the medium.
3. **DO NOT AUTOCLAVE.** Use immediately.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and off-white.

Prepared Appearance: Prepared medium is clear, very pale yellow with no or very slight precipitate.

Expected Cultural Response: Cultural response is exhibited on MacConkey Agar after enrichment in Selenite Cystine Broth at 35°C for 24 - 48 hours.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 11775	partial to complete inhibition	pink with bile precipitate
<i>Salmonella typhi</i> ATCC® 19430	good growth	colorless colony
<i>Salmonella typhimurium</i> ATCC® 14028	good growth	colorless colony
<i>Shigella sonnei</i> ATCC® 25931	fair to good growth	colorless colony

The organisms listed are the minimum that should be performed for quality control testing.

Test Procedure ^{2,3}

1. Prepare food sample following the recommended procedure.
2. Inoculate into recommended pre-enrichment broth.
3. Transfer 1 mL of mixture to 10 mL Selenite Cystine Broth and to 10 mL Tetrathionate Broth.
3. Incubate at 35°C for 24 ± 2 hours.
4. Mix and streak 3 mm loopful (10 µL) of sample from both broths onto Bismuth Sulfite Agar, Xylose Lysine Desoxycholate Agar, Hektoen Enteric Agar or MacConkey Agar.
5. Examine plates for the presence of colonies that are typical for *Salmonella* spp.

Results

Refer to references for the characteristic growth of *Salmonella* spp. on appropriate media formulations.

Storage

Store sealed container 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Selenite Cystine Broth	Code No.	7283A	500 g
		7283B	2 kg
		7283C	10 kg

References

1. **Leifson, E.** 1939. New selenite selective enrichment medium for the isolation of typhoid and paratyphoid bacilli. Am. J. Hyg. **24**:423-432.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual. 8th ed. AOAC International, Gaithersburg, MD.
3. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
4. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd. The United States Pharmacopeial Convention. Rockville, MD.
5. **Vanderzant, C. and D.F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

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SIM MEDIUM (7221)

Intended Use

SIM Medium is used for the differentiation of microorganisms on the basis of hydrogen sulfide production, indole production, and motility.

Product Summary and Explanation

Semisolid media have been used extensively in the determination of bacterial motility.¹ The production of hydrogen sulfide, indole formation, and motility are useful diagnostic tests in the identification of *Enterobacteriaceae*, especially *Salmonella* spp. and *Shigella* spp.

In 1940, Sulkin and Willett² demonstrated motility, hydrogen sulfide production, and carbohydrate fermentation by members of *Salmonella* and *Shigella* groups. They called attention to the “brush-like growth” or motility of the typhoid organisms. Greene et al. used SIM Medium to detect motility in a large series of cultures of typhoid organisms.³

Principles of the Procedure

The nitrogen, carbon, and amino acids sources in SIM Medium are provided by Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue. Ferric Ammonium Citrate and Sodium Thiosulfate are used to detect hydrogen sulfide production. H₂S gas reacts with Ferric Ammonium Citrate to produce ferrous sulfide, a black precipitate. SIM Medium is a semi-solid, due to the low concentration of agar. The semi-solid nature of this medium allows for easy visual determination of motility which appears as growth extending outward from the original line of inoculation.

Enzymatic Digest of Casein contains tryptophane, which is converted to indole. Indole is detected after incubation by the addition of Kovac's Reagent.

Formula / Liter

Enzymatic Digest of Casein	20 g
Enzymatic Digest of Animal Tissue.....	6.1 g
Ferric Ammonium Citrate.....	0.2 g
Sodium Thiosulfate	0.2 g
Agar	3.5 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Dissolve 30 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is light beige and clear to trace hazy.

Expected Cultural Response: Cultural response in SIM Medium at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions		
		Indole	Motility	H ₂ S
<i>Escherichia coli</i> ATCC® 25922	growth	positive	positive	positive
<i>Salmonella typhimurium</i> ATCC® 14028	growth	negative	positive	positive
<i>Shigella flexneri</i> ATCC® 12022	growth	negative	negative	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Using a wire, inoculate test organism two-thirds into the medium with stab motion.
2. Incubate with loose caps at 35 ± 2°C for 18 – 24 hours.
3. Examine tubes after incubation for motility and H₂S production.
4. Add 3 – 4 drops of Kovac's Reagent to each tube. Record as indole positive if a pink or red color appear, or as indole negative if there is no color change. Add Kovac's Reagent after determining motility and H₂S production.

Results

Motility is indicated by turbidity of the medium or growth extending from inoculating stab line. H₂S production is shown by a blackening along the stab line. Indole production is seen as the production of a red color after the addition of Kovac's Reagent. Indole is produced from the tryptophane present in the medium.

Refer to appropriate references for complete identification of *Enterobacteriaceae*.⁴

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. Avoid inoculum from liquid or broth suspension as growth initiation will be delayed.⁵
3. Reactions are not sufficient to speciate organisms. Additional biochemical and serological tests are required for confirmation.⁶

Packaging

SIM Medium	Code No.	7221A	500 g
		7221B	2 kg
		7221C	10 kg

References

1. **Tittsler, R. P. and L. A. Sandholzer.** 1936. The use of semi-solid agar for the detection of bacteria motility. J. Bact. **31**:575.
2. **Sulkin and Willett.** 1940. J. Lab Clin. Med. **25**:649.
3. **Greene, R. A., E. F. Blum, C. T. Decoro, R. B. Fairchild, M. T. Kapla, J. L. Landau, and T. R. Sharp.** 1951. Rapid method for the detection of motility. J. Bact. **62**:347.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover. (eds.).** 1995. Manual of clinical microbiology. 6th ed. American Society for Microbiology, Washington, D.C.
5. **Sosa.** 1943. Rev. Inst. Bact. **11**:286.
6. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SIMMONS CITRATE AGAR (7156)

Intended Use

Simmons Citrate Agar is used for the differentiation of microorganisms on the basis of citrate utilization.

Product Summary and Explanation

Koser first developed a liquid medium for differentiating coliforms from fecal coliforms. Fecal coliforms were unable to use citrate as the sole source of carbon and inorganic ammonium salt as a sole source of nitrogen. Non-fecal coliforms, such as *Enterobacter aerogenes* could use citrate in such a medium with resultant alkalinity. Liquid medium had the disadvantage of appearing turbid when a large inoculum was used, although no growth had taken place. This observation led Simmons to devise a solid medium that eliminated the problem with turbidity.²

Simmons Citrate Agar is a modification of Koser's medium, with the addition of bromthymol blue and 1.5% agar. Organisms able to metabolize citrate grow luxuriantly. The medium is alkalized and changes from green to deep blue in 24 – 48 hours. *Escherichia coli* either do not grow at all on this medium, or grow so sparsely that no change in reaction is apparent. Simmons Citrate Agar is recommended for differentiation of enteric gram-negative bacilli from clinical specimens,^{3,4} water samples,⁵ and food samples.^{6,7}

Principles of the Procedure

Ammonium Dihydrogen Phosphate is the sole source of nitrogen in Simmons Citrate Agar. Dipotassium Phosphate acts as a buffer. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate is the sole source of carbon in this medium. Magnesium Sulfate is a cofactor for a variety of metabolic reactions. Bromthymol Blue is the pH indicator. Organisms that can utilize Ammonium Dihydrogen Phosphate and Sodium Citrate as their sole sources of nitrogen and carbon will grow on this medium and produce a color change from green (neutral) to blue (alkaline). Agar is the solidifying agent.

Formula / Liter

Ammonium Dihydrogen Phosphate	1 g
Dipotassium Phosphate	1 g
Sodium Chloride	5 g
Sodium Citrate	2 g
Magnesium Sulfate	0.2 g
Bromthymol Blue.....	0.08 g
Agar	15 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 24.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into tubes and autoclave at 121°C for 15 minutes.
4. After autoclaving, allow medium to solidify in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and yellow with a green tint to green.

Prepared Appearance: Prepared medium is forest green and clear to trace hazy.

Expected Cultural Response: Cultural response on Simmons Citrate Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	blue colonies and medium
<i>Escherichia coli</i> ATCC® 25922	partial inhibition	green colonies and medium
<i>Salmonella choleraesuis</i> ATCC® 13076	partial inhibition	blue colonies and medium
<i>Salmonella typhimurium</i> ATCC® 14028	partial inhibition	blue colonies and medium
<i>Shigella flexneri</i> ATCC® 12022	inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Obtain a pure colony of the test organism.
2. Streak only the surface of the slant with a light inoculum.
3. Incubate tubes at 35 ± 2°C for 18 – 48 hours with loose caps.

Results

A positive reaction is indicated by growth on the slant with an intense blue color (alkaline reaction). A negative reaction is indicated by inhibition to poor growth without change in color (medium remains green).

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Some citrate positive organisms require a 48 hour incubation or longer for a pH change to occur.⁸

Packaging

Simmons Citrate	Code No.	7156A	500 g
		7156B	2 kg
		7156C	10 kg

References

1. **Koser, S. A.** 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bacteriol. **8**:493.
2. **Simmons, J. S.** 1926. A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. J. Infect. Dis. **39**:209.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. **Baron, E. J., L. R. Peterson, S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **FDA Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, MD.
8. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SKIM MILK (7352)

Intended Use

Skim Milk is dehydrated skim milk for use in preparing microbiological culture media.

Product 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 is added to enhance growth of *Mycobacterium tuberculosis* and *Corynebacterium diphtheria* in culture media. Skim Milk is used for differentiating organisms based on coagulation and proteolysis of casein in foods¹ and dairy products.² Indicators of pH, Litmus and Bromcresol Purple, are used with Skim Milk to determine acid production. Methylene Blue and Resazurin may be used with Skim Milk as oxidation-reduction indicators.

Principles of the Procedure

Skim Milk is a source of lactose and casein in prepared culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Expected Cultural Response: Cultural response as Litmus Milk at 35°C after 48 - 72 hours incubation.

Microorganism	Response	Reactions
<i>Bacillus subtilis</i> ATCC® 9372	growth	digested curd
<i>Clostridium perfringens</i> ATCC® 13124	growth	stormy fermentation, acid
<i>Escherichia coli</i> ATCC® 25922	growth	solid curd, acid
<i>Lactobacillus casei</i> ATCC® 393	growth	soft curd, acid, reduction
<i>Salmonella typhi</i> ATCC® 19430	growth	reduction

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Skim Milk.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Skim Milk at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Skim Milk should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to Skim Milk in its intact container when stored as directed.

Limitation of the Procedure

Skim Milk supports the growth of many microorganisms. Perform microscopic examination and other biochemical tests to identify isolates to the genus and species level.

Packaging
Skim Milk

Code No.	7352A	500 g
	7352B	2 kg
	7352C	10 kg

References

1. **Lee, J. S., and A. A. Kraft.** 1992. Proteolytic microorganisms, p. 193-198. *In* Vanderzant, C. and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Frank, J. F., G. L. Christen, and L. B. Bullerman.** 1993. Tests for groups of microorganisms, p. 271-286. *In* Marshall, R. T. (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

SOY PEPTONE YEAST EXTRACT AGAR (7224)

Intended Use

Soy Peptone Yeast Extract Agar is used for the isolation of dermatophytes.

Product Summary and Explanation

Soy Peptone Yeast Extract Agar is a modification of Sabouraud Agar, formulated by Carmichael and Claus.^{1,2} Soy Peptone Yeast Extract Agar was originally developed for the selective isolation of *Trichophyton verrucosum*, a fungus associated with ringworm infections. Ringworm is a fungal infection of the keratinized tissues including hair, nails, and skin of humans and lower animals caused by dermatophytes.³ Dermatophytes are composed of three genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*.³

Principles of the Procedure

Enzymatic Digest of Soybean Meal provides nitrogen, carbon, and amino acids required for organism growth in Soy Peptone Yeast Extract Agar. Yeast Extract supplies essential vitamins. Dextrose is included as an energy source. Streptomycin and Chloramphenicol are the selective agents, inhibiting most gram-positive and gram-negative bacteria, but allowing the detection of fungal pathogens. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Soybean Meal	10 g
Yeast Extract.....	5 g
Dextrose.....	40 g
Streptomycin	0.03 g
Chloramphenicol	0.05 g
Agar	17 g

Final pH: 6.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. VERY TOXIC. Toxic by inhalation and contact with skin.

Directions

1. Suspend 72 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and pale yellow-white.

Expected Cultural Response: Cultural response on Soy Peptone Yeast Extract Agar at 25- 30°C after 2 - 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Trichophyton mentagrophytes</i> ATCC® 9533	growth
<i>Trichophyton verrucosum</i> ATCC® 38485	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of dermatophytes, refer to appropriate references.^{3,4}

Results

Refer to appropriate references for results.^{3,4}

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Complete classification of dermatophytes is dependent upon microscopic observations of direct and slide culture preparations, along with biochemical and serological tests.
2. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Soy Peptone Yeast Extract Agar	Code No.	7224A	500 g
		7224B	2 kg
		7224C	10 kg

References

1. **Carmichael.** 1959. Alberta Med. Bull. **24**:201.
2. **Claus.** 1961. Mycopathol. **14**:129.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

STANDARD METHODS AGAR (7157)

Intended Use

Standard Methods Agar is used for the enumeration of bacteria in water, wastewater, food, and dairy products. This formula conforms to American Public Health Association¹ (APHA), and Association of Official Analytical Chemists² (AOAC).

Product Summary and Explanation

Standard Methods Agar was developed by Buchbinder, Baris, and Goldstein³ in 1953 at the request of the American Public Health Association. Results showed that a dehydrated milk-free medium containing 0.25% Yeast Extract, 0.5% Tryptone, 0.1% Dextrose, and 1.5% Agar per liter approximated the productivity of Tryptone Glucose Extract Agar with added milk. Buchbinder et al.⁴ recommended that a dehydrated culture medium be used in preparing the standard plate count medium rather than preparing the medium from ingredients. Standard Methods Agar as originally suggested by Buchbinder et al.⁴

Standard Methods Agar is also referred to as Plate Count Agar and Tryptone Glucose Yeast Agar. This formula is specified in standard method procedures.^{1,2,5-7}

Principles of the Procedure

Enzymatic Digest of Casein and Yeast Extract provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Dextrose is a source of fermentable carbohydrate (energy source). Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein 5 g
Yeast Extract..... 2.5 g
Dextrose..... 1 g
Agar 15 g

Final pH: 7.0 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 23.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and light beige to medium amber.

Expected Cultural Response: Cultural response on Standard Methods Agar at 35°C after 18 - 24 hours incubation.

Test Sample	Response
Unpasteurized (raw) milk	Excellent growth and recovery w/ a t-value < 2.70

Test Procedure

1. Perform serial dilutions on samples (food, water) to be tested using the heterotrophic (standard) plate count method. Select dilutions that will yield plates with counts of 30 - 300 colonies.
2. Dispense a portion of each test dilution (e.g., 0.1 mL, 1.0 mL) into separate test dilutions.
3. Add 10 - 12 mL of tempered (45°C) Standard Methods Agar to petri dishes containing test dilutions.
4. Swirl the dishes to thoroughly mix the agar and test dilution.
5. Allow plates to cool and solidify.
6. Incubate at $32 \pm 1^\circ\text{C}$ for 48 hours.

Results

Count colonies on all plates containing 30 - 300 colonies. Calculate bacterial count per milliliter of sample by multiplying the average number of colonies per plate by the reciprocal of the dilution used. Report the count as CFU/mL.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Standard Methods Agar	Code No.	7157A	500 g
		7157B	2 kg
		7157C	10 kg

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Cunniff, P. (ed.).** 1995. Official methods of analysis AOAC International, 16th ed. AOAC International, Arlington, VA.
3. **Buchbinder, L., Y. Baris, and L. Goldstein.** 1953. Further studies on new milk-free media for the standard plate count of dairy products. Am J. Public Health **43**:869-872.
4. **Buchbinder, L., Y. Baris, E. Alff, E. Reynolds, E. Dillon, V. Pessin, L. Pincus, and A. Strauss.** 1951. Studies to formulate new media for the standard plate count of dairy products. Pub. Health Rep. **66**:327-340.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.).** 1992. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington, D.C.
7. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

STAPH SELECTIVE AGAR (7373)

Intended Use

Staph Selective Agar is used for the selective isolation of *Staphylococcus* spp.

Product Summary and Explanation

Staph Selective Agar is a medium used for the selective isolation of pathogenic staphylococci. Chapman formulated Mannitol Salt Agar to isolate staphylococci by inhibiting growth of most other bacteria with a high salt concentration.¹ The formulation of Staph Selective Agar contains a lower concentration of salt than Mannitol Salt Agar. The lower salt concentration appears to enhance the growth of most staphylococci. Selective agents are added to improve the inhibition of organisms other than pathogenic staphylococci.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide nitrogen, vitamins, and carbon in Staph Selective Agar. D-Mannitol is the carbohydrate source. The high concentration of Sodium Chloride and the addition of selective agents inhibit most gram-negative bacteria and non-pathogenic staphylococci. Bromcresol Purple is the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Beef Extract	2 g
D-Mannitol.....	10 g
Sodium Chloride	40 g
Selective Agents	14 g
Bromcresol Purple	0.02 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful if swallowed, inhaled, or absorbed through skin.

Directions

1. Suspend 101 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light bluish - beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and purple.

Expected Cultural Response: Cultural response on Staph Selective Agar at 35°C after 24 - 48 hours incubation.

Microorganism	Response	Reaction
<i>Escherichia coli</i> ATCC® 25922	inhibited	N/A
<i>Staphylococcus aureus</i> ATCC® 25923	growth	yellow colonies
<i>Staphylococcus epidermidis</i> ATCC® 12228	partially inhibited	colorless, translucent colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate specimen on medium for primary isolation, or inoculate isolated colonies onto medium for differentiation. For a complete discussion on staphylococci, refer to appropriate references.^{2,3}

Results

Staphylococci will grow on this medium, while growth of most other bacteria will be inhibited. Pathogenic staphylococci produce yellow colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Staph Selective Agar	Code No.	7373A	500 g
		7373B	2 kg
		7373C	10 kg

References

1. **Chapman, G. H.** The significance of sodium chloride in studies of staphylococci. J. Bacteriol. **50**:201.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Isenberg, H. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

STAPHYLOCOCCUS AGAR # 110 (7158)

Intended Use

Staphylococcus Agar # 110 is used for the isolation of staphylococci.

Product Summary and Explanation

Stone described a culture medium where staphylococci food-poisoning produced a positive gelatinase test.¹ Chapman, Lieb, and Curcio later reported pathogenic staphylococci strains typically ferment mannitol, form pigment, and produce gelatinase.² Chapman suggested adding 7.5% NaCl to Phenol Red Mannitol Agar, which led to the development of Staphylococcus Agar # 110.^{3,4} The high salt concentration contributes to the selective isolation of pathogenic staphylococci. Staphylococcus Agar # 110 is included in standard methods procedures for selectively isolating pathogenic staphylococci from food.⁵

Staphylococcus Agar # 110 is also known as Staphylococcus Medium No. 110 and Stone Gelatin Agar.⁶

Principles of the Procedure

Enzymatic Digest of Casein is the source of nitrogen, carbons, and minerals in Staphylococcus Agar # 110. Gelatin is included for testing liquefaction. Yeast Extract supplies B-complex vitamins to stimulate bacterial growth. Sodium Chloride, in high concentration, inhibits most bacteria other than staphylococci. Lactose and D-Mannitol are the carbohydrates. Dipotassium Phosphate buffers the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Gelatin.....	30 g
Yeast Extract.....	2.5 g
Lactose	2 g
D-Mannitol.....	10 g
Sodium Chloride	75 g
Dipotassium Phosphate.....	5 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 149 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, and yellow-beige.

Expected Cultural Response: Cultural response on Staphylococcus Agar # 110 at 35°C after 24 - 48 hours incubation.

Microorganism	Response	Reactions	
		Mannitol	Gelatin
<i>Escherichia coli</i> ATCC® 25922	inhibited	---	---
<i>Proteus mirabilis</i> ATCC® 12453	growth	negative	negative
<i>Staphylococcus aureus</i> ATCC® 25923	growth	positive	positive
<i>Staphylococcus epidermidis</i> ATCC® 12228	growth	negative	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Mannitol fermentation is indicated by a color change of bromthymol blue or bromcresol purple (0.04% in 50:50 ethanol: distilled water) after placing a drop of the dye onto the agar surface where colonies have been removed.
2. Gelatin hydrolysis is determined by flooding the plate with 5 mL of a saturated aqueous solution of ammonium sulfate and incubating plates at 35°C for 10 minutes.

Results

Growth of pathogenic staphylococci produce yellow to orange pigmented colonies. Perform additional biochemical tests on suspected colonies for complete identification of staphylococci.

Gelatin hydrolysis is indicated by a clear zone around the colonies after flooding the medium with ammonium sulfate.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. *Enterococcus faecalis* may grow on Staphylococcus Agar # 110 as tiny colonies with mannitol fermentation.
2. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Staphylococcus Agar # 110	Code No.	7158A	500 g
		7158B	2 kg
		7158C	10 kg

References

1. **Stone, R. V.** 1935. A cultural method for classifying staphylococci as of the "food poisoning" type. Proc. Soc. Exptl. Biol. Med. **33**:185-187.
2. **Chapman, G. H., C. W. Lieb, and L. G. Curcio.** 1937. Isolation and cultural differentiation of food-poisoning staphylococci. Food Research. **2**:349.
3. **Chapman, G. H.** 1945. The significance of sodium chloride in studies of staphylococci. J. Bacteriol. **50**:201.
4. **Chapman, G. H.** 1946. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation and the Stone reaction. J. Bacteriol. **51**:409.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **MacFaddin, J. F.** 1985. Media for isolating-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 722-726. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

STERILITY TEST BROTH (7159) (USP Alternate Thioglycollate Medium)

Intended Use

Sterility Test Broth is used in sterility testing.

Product Summary and Explanation

Quastel and Stephenson found the presence of small amounts of a compound containing an –SH group (cystein, thioglycollic acid, and glutathione) permitted “aerobic” growth of *Clostridium sporogenes*.¹ Falk, Bucca, and Simmons discovered the advantages of using small quantities of agar in detecting contaminants during sterility testing.² Brewer demonstrated the value of a small amount of agar and a reducing substance in this medium.³

Sterility Test Broth, also referred to as USP Alternate Thioglycollate Medium, is used to detect microorganisms in normally sterile, turbid, or viscous materials. Sterility Test Broth has also been called NIH Thioglycollate Broth.

Principles of the Procedure

Sterility Test Broth supports the growth of a large variety of fastidious microorganisms having a wide range of growth requirements. The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Yeast Extract. Dextrose is the carbohydrate energy source, and Sodium Chloride maintains the osmotic balance of the medium. Sodium Thioglycollate and L-Cystine lower the oxidation-reduction potential of the medium by removing oxygen to maintain a low Eh. By creating an environment with a low Eh, the reducing agents prevent the accumulation of peroxides that can be toxic to some organisms. The sulfhydryl groups (–SH) of these compounds also neutralize the antibacterial effect of mercurial preservatives, making thioglycollate media useful in testing material containing heavy metals.

Formula / Liter

Enzymatic Digest of Casein	15 g
Yeast Extract.....	5 g
Dextrose.....	5.5 g
Sodium Chloride	2.5 g
Sodium Thioglycollate.....	0.5 g
L-Cystine	0.5 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 29 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for a few minutes or until solution occurs.
3. Dispense into tubes and autoclave at 121°C for 15 minutes. Cool to room temperature.

NOTE: Unless used on the same day of preparation, the prepared tubes should be heated in boiling water bath or steam bath, and cooled before use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear or hazy, and light amber in color.

Expected Cultural Response: Cultural response at 35°C after 1 – 7 days incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 6633	growth
<i>Bacteroides vulgatus</i> ATCC® 8482	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Clostridium sporogenes</i> ATCC® 11437	growth
<i>Micrococcus luteus</i> ATCC® 9341	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Sterility Test Broth.

Results

Typically growth is visually observed in the media. Gram-negative bacilli usually grow diffusely, Gram-positive cocci exhibit puff-ball type growth and strict aerobes, such as pseudomonads and yeast, grow in a thin layer on the surface of the broth.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Sterility Test Broth	Code No.	7159A	500 g
		7159B	2 kg
		7159C	10 kg

References

1. **Quastel and Stephenson.** 1926. General biological product standards. Fed. Regist. **21**:6109-12.
2. **Falk, C. R., H. Bucca, and M. P. Simmons.** 1939. A comparative study of the use of varying concentrations of agar in the test medium used to detect contaminants in biological products. J. Bacteriol. **37**:121-131.
3. **Brewer, J. H.** 1940. Clear liquid mediums for the "aerobic" cultivation of anaerobes. J. Amer. Med. Assoc. **115**:598-600.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TAT BROTH (7219)

Intended Use

TAT Broth is used for the detection of microorganisms in cosmetics and topical drugs.

Product Summary and Explanation

TAT Broth is prepared according to the formula obtained from the U. S. Food and Drug Administration for use in the examination of cosmetics and topical drugs.¹ TAT Broth, with the addition of Polysorbate 20, is recommended for sterility testing of viscous materials, including salves or ointments. It is especially adapted for sterility testing of cosmetics. Cosmetics and pharmaceutical products are subject to contamination during manufacturing and use by consumers.² Preservatives are used in aqueous products to make them self-sterilizing for vegetative bacteria, yeast, and molds.²

TAT (Tryptone-Azolectin-Tween) Broth is also referred to as Fluid Casein Digest-Soy Lecithin Polysorbate 20 Medium.

Principles of the Procedure

The nitrogen, vitamins, and carbon sources are provided by Enzymatic Digest of Casein in TAT Broth. Lecithin and Polysorbate 20 neutralize preservatives in cosmetics or pharmaceutical products, allowing bacteria to grow.

Formula / Liter

Enzymatic Digest of Casein 20 g

Lecithin..... 5 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Polysorbate 20, 40 mL

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 25 g of the medium in 960 mL of purified water until evenly dissolved.
2. At room temperature, add 40 mL of polysorbate 20 to the suspended medium.
3. Place the mixture in a 48 - 50°C waterbath for 30 minutes and stir occasionally to dissolve.
4. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and off-white.

Prepared Appearance: Prepared medium is clear to slightly hazy and light yellow.

Expected Cultural Response: Cultural response in TAT Broth at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth
<i>Salmonella typhi</i> ATCC® 19430	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Add one gram or one mL of an undiluted sample to 40 mL of complete medium and agitate to obtain an even suspension.
2. Incubate tubes at $35 \pm 2^{\circ}\text{C}$ for 18 – 48 hours.

For a complete discussion on sterility testing refer to appropriate procedures in USP.³

Results

Tubes or bottles exhibiting growth should be subcultured for identification.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

TAT Broth	Code No.	7219A	500 g
		7219B	2 kg
		7219C	10 kg

References

1. **Orth, D. S.** 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, N.Y.
2. **Food and Drug Administration.** 1969. Procedure for the examination of topical drugs and cosmetics. FDA, Rockville, MD.
3. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. Microbial limits tests, p. 1681-1686. The United States Pharmacopeial Convention Inc., Rockville, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TCBS AGAR (7210)

Intended Use

TCBS Agar is used for the selective isolation of *Vibrio cholerae* and other enteropathogenic vibrios.

Product Summary and Explanation

TCBS Agar is an abbreviation for Thiosulfate-Citrate-Bile-Sucrose Agar. TCBS Agar is also called Vibrio Selective Agar, and prepared according to the formula of Kobayashi et al.¹ This formula is a modification of the selective medium from Nakanishi.² All pathogenic *Vibrio* spp., except *Vibrio hollisae*, will grow on TCBS Agar. This highly selective agar meets the nutritional requirements of *Vibrio* spp., and allows vibrios to compete with intestinal flora. *Vibrio* spp. are able to grow in media containing increased salt concentrations, and some species are halophilic.³ Infections have been associated with ingestion of contaminated water and consumption of contaminated shellfish or seafood. *Vibrio* spp. are natural inhabitants of seawater.³

TCBS Agar is recommended for isolating *Vibrio* spp. from stool specimens,⁴ and specified in standard methods for food testing.^{5,6}

Principles of the Procedure

Yeast Extract, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue provides the nitrogen, vitamins, and amino acids in TCBS Agar. Sodium Citrate, Sodium Thiosulfate, Sodium Cholate, and Oxbile are selective agents, providing an alkaline pH to inhibit Gram-positive organisms and suppress coliforms. An increased pH is used to enhance growth of *Vibrio cholerae*, because this organism is sensitive to acid environments. Sucrose is the fermentable carbohydrate. Sodium Chloride stimulates organism growth and maintains osmotic balance of the medium. Sodium Thiosulfate is also a sulfur source, and acts with Ferric Citrate as an indicator to detect hydrogen sulfide production. Bromthymol Blue and Thymol Blue are pH indicators. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	5 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Sodium Citrate	10 g
Sodium Thiosulfate	10 g
Oxbile.....	5 g
Sodium Cholate	3 g
Sucrose.....	20 g
Sodium Chloride	10 g
Ferric Citrate	1 g
Bromthymol Blue.....	0.04 g
Thymol Blue	0.04 g
Agar	14 g

Final pH: 8.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 88 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to green-beige.

Prepared Appearance: Prepared medium is forest green and clear to trace hazy.

Expected Cultural Response: Cultural response on TCBS Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	---
<i>Escherichia coli</i> ATCC® 25922	inhibited	---
<i>Vibrio alginolyticus</i> ATCC® 17749	growth	yellow colonies
<i>Vibrio cholerae</i> ATCC® 9459	growth	yellow colonies
<i>Vibrio parahaemolyticus</i> ATCC® 17802	growth	green colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of *Vibrio cholerae* and other enteropathogenic vibrios, refer to specific procedures.

Results

After 18 – 24 hours of incubation at 35°C, sucrose-fermentating vibrios (*V. cholerae*, *V. alginolyticus*, *V. hareyi*, *V. cincinnatiensis*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, and some *V. vulnificus*) appear smooth, opaque, thin-edged yellow colonies on TCBS Agar.³

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Vibrio* spp.
2. On initial isolation, *V. parahaemolyticus* may be confused with *Aeromonas hydrophila*, *Plesiomonas shigelloides*, and *Pseudomonas* spp.⁷
3. Sucrose-fermenting *Proteus* spp. produce yellow colonies which may resemble those of *Vibrio*.⁸
4. TCBS is an unsatisfactory medium for oxidase testing of *Vibrio* spp.⁹
5. A few strains of *V. cholerae* may appear green or colorless on TCBS Agar due to delayed sucrose fermentation.⁸

Packaging

TCBS Agar	Code No.	7210A	500 g
		7210B	2 kg
		7210C	10 kg

References

1. Kobayashi, T., S. Enomoto, R. Sakazaki, and S. Kuwahara. 1963. A new selective medium for pathogenic vibrios, TCBS (modified Nakanishi's agar). Jpn. J. Bacteriol. 18:387.
2. Nakanishi, Y. 1963. An isolation agar medium for cholerae and enteropathogenic halophilic vibrios. Modern Media. 9:246.
3. Baron, E. J., L. R. Peterson, and S. M. Finegold. 1994. *Vibrio* and related species, *Aeromonas*, *Plesiomonas*, *Campylobacter*, *Helicobacter*, and others, p. 429-444. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
4. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. Vanderzant, C. and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of food. 3rd ed. American Public Health Association, Washington, D.C.
7. Bottone, E. J., and T. Robin. 1978. *Vibrio parahaemolyticus*; Suspicion of presence based on aberrant biochemical and morphological features. J. Clin. Microbiol. 8:760.
8. MacFaddin, J. D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1, p. 763-767. Williams & Wilkins, Baltimore, MD.
9. Morris, G. K., M. H. Merson, I. Huq, A. Kibria, and R. Black. 1979. Comparison of four plating media for isolating *Vibrio cholerae*. J. Clin. Microbiol. 9:79.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TERGITOL 7 AGAR (7187)

Intended Use

Tergitol 7 Agar is used for the selective isolation of coliform bacteria.

Product Summary and Explanation

Tergitol 7 Agar, prepared according to the formula published by Chapman, is selective for *Escherichia coli* and members of the coliform group.¹ Chapman reported that the addition of Tergitol 7 to a medium consisting of polypeptone and yeast extract permitted unrestricted development of all coliform bacteria, and inhibited gram-negative spore-formers and Gram-positive organisms. Coliform counts on Tergitol 7 Agar were 30% higher than on other selective media.

Chapman modified his original Tergitol 7 Agar by adding 40 mg of triphenyltetrazolium chloride (TTC) per liter.² This medium was helpful in the early recognition and identification of *Escherichia coli*. Confirmation of the presence of *E. coli* was possible after only 10 hours incubation at 35°C. Chapman also reported that Tergitol 7 Agar with added TTC gave a selective medium suitable for the isolation of *Candida* spp. and other fungi. Tergitol 7 Agar with TTC is useful for routine water analysis and the examination of foods.^{3,5}

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are the nitrogen and mineral sources in Tergitol 7 Agar. Yeast Extract supplies essential vitamins. Lactose is the fermentable carbohydrate. Lactose fermentation is indicated by a color change of the pH indicator, Bromthymol Blue. Tergitol 7 (sodium heptadecyl sulfate) inhibits growth of gram-positive microorganisms, spore-forming gram-negative bacteria, and swarming of *Proteus* spp. Agar is the solidifying agent.

When TTC is added to the medium, it serves as an indicator of bacterial growth. TTC is rapidly reduced to insoluble red formazan by most organisms. Lactose fermenting organisms continue to provide yellow to greenish-yellow colonies. Non-lactose fermenters appear red due to uptake and reduction of the TTC.

Formula / Liter

Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Yeast Extract.....	3 g
Lactose	10 g
Tergitol 7	0.1 g
Bromthymol Blue.....	0.025 g
Agar	15 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 33 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

OPTIONAL: Cool Tergitol 7 Agar to 50°C. Add 4 mL of either TTC Solution 1% or a filter sterilized 1% solution of TTC.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light green-beige to light beige.

Prepared Appearance: Prepared medium is green and trace to slightly hazy.

Expected Cultural Response: Cultural response on Tergitol 7 Agar at 35°C after 12 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	yellow colonies
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	---
<i>Escherichia coli</i> ATCC® 25922	growth	yellow colonies
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	---
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	blue colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	blue colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

In the absence of TTC, *E. coli* produces yellow colonies with yellow halos; other coliforms produce yellow to yellow-green colonies. Non-fermenters produce blue colonies. With added TTC, *E. coli* produces yellow colonies; other coliforms produce yellow-green colonies, while non-fermenters produce red colonies.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Pour plates do not give satisfactory results.
3. Allow plates to dry with lids slightly ajar for 1 – 2 hours.⁶
4. Reduction of TTC is an irreversible reaction that produces an insoluble formazan compound.

Packaging

Tergitol 7 Agar	Code No.	7187A	500 g
		7187B	2 kg
		7187C	10 kg

References

1. **Chapman, G. H.** 1947. A superior culture medium for the enumeration and differentiation of coliforms. J. Bacteriol. **53**:504.
2. **Chapman, G. H.** 1951. A culture medium for detecting and confirming *Escherichia coli* in ten hours. Am. J. Public Health. **41**:1381.
3. **Kulp, W., C. Mascoli, and O. Tavshanjian.** 1953. Use of tergitol-7 triphenyl tetrazolium chloride agar as the coliform confirmatory medium in routine sanitary water analysis. Am. J. Public Health. **43**:1111.
4. **Mossel, D. A. A.** 1962. An ecological investigation on the usefulness of two specific modifications of Eijkman's test as an element of the methods for the detecting of faecal contamination of food. J. Appl. Bacteriol. **25**:20.
5. **Speck, Marvin L. (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TETRATHIONATE BROTH BASE (7241)

Intended Use

Tetrathionate Broth Base is used with iodine for the recovery of *Salmonella* spp.

Product Summary and Explanation

Tetrathionate Broth Base is used as a selective enrichment for the cultivation of *Salmonella* spp. 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, preservative, and sanitizers.¹ *Salmonella* spp. cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.²

Mueller³ demonstrated the effectiveness of Tetrathionate Broth for enriching typhoid and paratyphoid bacilli while inhibiting coliform organisms. Using modified Mueller's broth, Kauffmann^{4,5} increased the number of positive isolates. Tetrathionate Broth was used in studies for the poultry industry^{6,7} and in a collaborative study for rapid screening of *Salmonella* in food.⁸ Tetrathionate Broth Base, abbreviated as TT Broth Base, is specified in standard methods⁹⁻¹² for *Salmonella* testing. Tetrathionate Broth Base is used in processing fecal cultures for bacteria.¹³

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provides nitrogen, carbon, vitamins, and amino acids in Tetrathionate Broth Base. Selectivity is accomplished by the combination of Sodium Thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.¹⁴ Tetrathionate is formed in the medium upon addition of the iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will proliferate in the medium. Bile Salts, a selective agent, suppresses coliform bacteria and inhibits Gram-positive organisms. Calcium Carbonate neutralizes and absorbs toxic metabolites.

Formula / Liter

Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Bile Salts	1 g
Calcium Carbonate	10 g
Sodium Thiosulfate	30 g

Final pH: 8.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful if swallowed or inhaled. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 46 g of the medium in one liter of purified water.
2. Heat with frequent agitation to boiling.
3. Cool to 45°C and add 20 mL of an iodine-iodide solution (6 grams iodine + 5 grams potassium iodide in 20 mL of purified water). DO NOT REHEAT AFTER ADDING IODINE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and white.

Prepared Appearance: Prepared medium is milky white and opaque.

Expected Cultural Response: Cultural response after enrichment in Tetrathionate Broth Base (with the iodine solution) and subcultured to MacConkey Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Salmonella arizonae</i> ATCC® 13314	good growth
<i>Salmonella typhimurium</i> ATCC® 14028	good growth
<i>Shigella flexneri</i> ATCC® 12022	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion of the isolation and identification of *Salmonella*, refer to appropriate references.

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Tetrathionate Broth Base	Code No.	7241A	500 g
		7241B	2 kg
		7241C	10 kg

References

1. Hartman, P. A., and S. A. Minnich. 1981. Automation for rapid identification of salmonellae in foods. J. Food Prot. **44**:385-386.
2. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. Mueller, L. 1923. Un Nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. C. R. Soc. Bio. **89**:434. Paris.
4. Kauffmann, F. 1930. Ein kombiniertes anreicherungsverfahren fur typhus und-paratyphusbacillen. Zentralb. Bakteriologie. Parasitenkunde. Infektionskrankheiten. Hygiene. Abt. I orig. **113**:148.
5. Kauffman, F. 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren fur Salmonella bacillen. Z. Hyg. Infektionskrankheiten. **117**:26.
6. Jones, F. T., R. C. Axtell, D. V. Rives, S. E. Scheideler, F. R. Tarver, Jr., R. L. Walker, and M. J. Wineland. 1991. A survey of *Salmonella* contamination in modern broiler production. J. Food Prot. **54**:502-507.
7. Barnhart, H. M., D. W. Dressen, R. Bastien, and O. C. Pancorbo. 1991. Prevalence of *Salmonella enteritidis* and other serovars in ovaries of layer hens at time of slaughter. J. Food Prot. **54**:488-492.
8. Eckner, K. F., W. A. Dustman, M. S. Curiale, R. S. Flowers, and B. J. Robison. 1994. Elevated-temperature, colorimetric, monoclonal, enzyme linked immunosorbent assay for rapid screening of *Salmonella* in foods; collaborative study. J. Assoc. Off. Anal. Chem. **77**:374-383.
9. Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
10. Marshall, R. T. (ed.). 1993. Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
11. United States Pharmacopeial Convention. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
12. Federal Register. 1991. Animal and plant health inspection service: chicken affected by *Salmonella enteritidis*, final rule, Fed. Regist. **56**:3730-3743.
13. Isenberg, H. D. (ed.). 1992 Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology. Washington, D. C.
14. Knox, R., P. H. Gell, and M. R. Pollack. 1942. Selective media for organisms of the *Salmonella* group. J. Pathol. Bacteriol. **54**:469-483.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

THIOGLYCOLLATE MEDIUM w/o INDICATOR (7160)

Intended Use

Thioglycollate Medium w/o Indicator is used for the cultivation of anaerobic microorganisms.

Product Summary and Explanation

Quastel and Stephenson found the presence of small amounts of a compound containing an –SH group (cysteine, thioglycolic acid, and glutathione) permitted “aerobic” growth of *Clostridium sporogenes*.¹ Falk, Bucca, and Simmons discovered the advantages of using small quantities of agar in detecting contaminants during sterility testing.² Brewer demonstrated the value of a small amount of agar and a reducing substance in this medium.³

Thioglycollate Medium w/o Indicator is used for cultivating and detecting microorganisms in normally sterile materials, especially those containing mercurial preservatives when the oxidation-reduction indicator is not present or required. Thioglycollate Medium w/o Indicator is the medium of choice for diagnostic testing, where lack of an indicator avoids possible toxicity to organisms.⁴

Principles of the Procedure

Thioglycollate Medium w/o Indicator supports the growth of a large variety of fastidious microorganisms having a wide range of growth requirements. The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal. Dextrose is the carbohydrate energy source, and Sodium Chloride maintains the osmotic balance of the medium. Sodium Thioglycollate and L-Cystine lower the oxidation-reduction potential of the medium by removing oxygen to maintain a low Eh. By creating an environment with a low Eh, the reducing agents prevent the accumulation of peroxides that can be toxic to some organisms. The sulfhydryl groups (-SH) of these compounds also neutralize the antibacterial effect of mercurial preservatives, making thioglycollate media useful in testing material containing heavy metals. Agar eliminates the need for seals because it retards dispersion of CO₂, diffusion of oxygen, and reducing substances.⁵

Formula / Liter

Enzymatic Digest of Casein	17 g
Enzymatic Digest of Soybean Meal	3 g
Dextrose.....	5.5 g
Sodium Chloride	2.5 g
L-Cystine	0.25 g
Sodium Thioglycollate	0.5 g
Agar	0.75 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Dissolve 29.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear or hazy, yellow, with upper 10% or less light to medium red upon standing.

Expected Cultural Response: Cultural response at 35°C after 1 – 7 days incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 6633	growth
<i>Bacteroides vulgatus</i> ATCC® 8482	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Clostridium sporogenes</i> ATCC® 11437	growth
<i>Micrococcus luteus</i> ATCC® 9341	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Thioglycollate Medium w/o Indicator.

Results

Typically growth is visually observed in the media. Gram-negative bacilli usually grow diffusely, gram-positive cocci exhibit puff-ball type growth and strict aerobes, such as pseudomonads and yeast, grow in a thin layer on the surface of the medium.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Thioglycollate Medium w/o Indicator	Code No.	7160A	500 g
		7160B	2 kg
		7160C	10 kg

References

1. Quastel and Stephenson. 1926. General biological products standards. Fed. Regist. **21**:6109-12.
2. Falk, C. R., H. Bucca, and M. P. Simmons. 1939. A comparative study of the use of varying concentrations of agar in the test medium used to detect contaminants in biological products. J. Bacteriol. **37**:121-131.
3. Brewer, J. H. 1940. Clear liquid mediums for the "aerobic" cultivation of anaerobes. J. Amer. Med. Assoc. **115**:598-600.
4. Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel. 1995. *Clostridium perfringens*, p. 16.01-16.06. In Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. MacFaddin, J. F. 1985. Media for isolation-cultivation-identification maintenance of medical bacteria, vol. 1, p. 755-762. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TODD HEWITT BROTH (7161)

Intended Use

Todd Hewitt Broth is used for the cultivation of streptococci and other fastidious microorganisms.

Product Summary and Explanation

Todd Hewitt Broth was originally developed for the production of antigenic streptococcal hemolysin.¹ Todd Hewitt Broth is prepared according to the formula described by Updyke and Nickle, who compared media for type specific extract production of group A streptococci.² This study was performed using Todd Hewitt Broth prepared with infusion of fresh beef heart as a control. Results showed Todd Hewitt Broth was particularly satisfactory for growth of group A streptococci for serological typing.

Moody et al. used Todd Hewitt Broth in the fluorescent-antibody identification of group A streptococci from throat cultures.³ Todd Hewitt Broth is recommended as an enrichment medium for growth of streptococcal cells in the identification of groups A and B by IF staining.⁴ Todd Hewitt Broth was used as an enrichment broth for group A streptococci in a comparison study of a rapid antigen test.⁵

Principles of the Procedure

The nitrogen source is provided by Heart Infusion in Todd Hewitt Broth. Yeast Enriched Peptone supplies vitamins and essential minerals. Dextrose is a carbon source and a stimulant for hemolysin production.⁶ Sodium Chloride maintains the osmotic balance of the medium. Disodium Phosphate and Sodium Carbonate act as buffers to aid in neutralizing acid production from dextrose fermentation and protect hemolysin from inactivation.⁶

Formula / Liter

Heart Infusion (dehydrated) 3.1 g
Yeast Enriched Peptone 20 g
Dextrose..... 2 g
Sodium Chloride 2 g
Disodium Phosphate..... 0.4 g
Sodium Carbonate 2.5 g

Final pH: 7.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Dissolve 30 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium gold-amber and clear to trace hazy.

Expected Cultural Response: Cultural response in Todd Hewitt Broth at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Enterococcus faecalis</i> ATCC® 29212	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation, identification, and serological procedures of streptococci and other fastidious microorganisms, refer to the procedures described in appropriate references.^{3,4,7}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2-30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to the expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Todd Hewitt Broth cannot be used unbuffered for bile solubility testing.⁶

Packaging

Todd Hewitt Broth	Code No.	7161A	500 g
		7161B	2 kg
		7161C	10 kg

References

1. **Todd, E. W., and L. F. Hewitt.** 1932. A new culture medium for the production of antigenic streptococcal haemolysin. J. Pathol. Bacteriol. **35**:973.
2. **Updyke, E. L., and M. I. Nickle.** 1954. A dehydrated medium for the preparation of type specific extracts of group A streptococci. Appl. Microbiol. **2**:117.
3. **Moody, M. D., A. C. Siegel, B. Pittman, and C. C. Winter.** 1963. Fluorescent-antibody identification of group A streptococci from throat swabs. Am. J. Public Health. **53**:1083.
4. **Facklam, R. R., and R. B. Carey.** 1985. *Streptococci and Aerococci*, p. 154-175. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (eds.). Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. **Bourbeau, P. P., B. J. Heiter, J. P. Anhalt, and D. W. Naumovitz.** 1993. Comparison of direct specimen testing utilizing testpack strep A with testing of specimens following a two-hour broth enrichment. Diagn. Microbiol. Infect. Dis. **17**:93-96.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification maintenance of medical bacteria, vol. 1, p. 755-762. Williams & Wilkins, Baltimore, MD.
7. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TOMATO JUICE AGAR (7349)

Intended Use

Tomato Juice Agar is used for the cultivation of lactobacilli.

Product Summary and Explanation

In 1925, Mickle and Breed reported the use of tomato juice in culture media used for cultivating lactobacilli.¹ Kulp investigated tomato juice on bacterial development and found growth of *L. acidophilus* was enhanced.² Tomato Juice Agar, prepared according to Kulp and White's³ modification, is especially useful in cultivating *L. acidophilus* from clinical specimens and foodstuffs.⁴

Principles of the Procedure

Tomato Juice Solids is a source of carbon, protein, and nutrients. Enzymatic Digest of Casein provides nitrogen, amino acids, and carbon used for general growth requirements. Peptonized Milk contains lactose as an energy source. Agar is the solidifying agent.

Formula / Liter

Tomato Juice Solids	20 g
Enzymatic Digest of Casein	10 g
Peptonized Milk.....	10 g
Agar	11 g

Final pH: 6.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 51 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and orange-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, and light to medium yellow-beige.

Expected Cultural Response: Cultural response on Tomato Juice Agar at 35°C after 24 - 72 hours incubation.

Microorganism	Response
<i>Lactobacillus casei</i> ATCC® 393	growth
<i>Lactobacillus fermentum</i> ATCC® 9338	growth
<i>Lactobacillus plantarum</i> ATCC® 8014	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Organisms other than lactobacilli may grow on this medium. Isolates must be confirmed by appropriate biochemical tests.

Packaging

Tomato Juice Agar	Code No.	7349A	500 g
		7349B	2 kg
		7349C	10 kg

References

1. **Mickle and Breed.** 1925. Technical Bulletin 110. NY State Agriculture Exp. Station.
2. **Kulp, W. L.** 1927. Scientific apparatus and laboratory methods. An agar medium for plating *L. acidophilus* and *L. bulgaricus*. Science. **66**:512-513.
3. **Kulp, W. L., and V. White.** 1932. Modified medium for plating *L. acidophilus*. Science. **76**:17-18.
4. **MacFaddin, J. D.** 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol 1, p. 776-778. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRIPLE SUGAR IRON AGAR (7162)

Intended Use

Triple Sugar Iron Agar is used for the differentiation of microorganisms on the basis of dextrose, lactose, and sucrose fermentation and hydrogen sulfide production.

Product Summary and Explanation

In 1911, Russell described the use of two sugars in a medium to differentiate gram-negative organisms of intestinal origin.¹ Lead or iron salts were added to Russell's medium to detect the presence of hydrogen sulfide. Kligler added lead acetate to Russell Double Sugar Agar, resulting in a medium capable of differentiating typhoid, paratyphoid, and dysentery.^{2,3} A modification of this medium was developed, Kligler Iron Agar, using Phenol Red as an indicator and iron salts to detect hydrogen sulfide production. In 1940, Sulkin and Willett described a triple sugar ferrous sulfate medium for use in identification of enteric organisms.⁴ Triple Sugar Iron Agar is essentially the formula originally described by Sulkin and Willett.⁴

Triple Sugar Iron Agar is recommended for differentiation of enteric, gram-negative bacilli from clinical specimens, dairy samples, and food products.⁵⁻⁷

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Yeast Enriched Peptone provide the nitrogen, carbon, and vitamins required for organism growth. Triple Sugar Iron Agar contains three carbohydrates, Dextrose, Lactose and Sucrose. When the carbohydrates are fermented, acid production is detected by the Phenol Red pH indicator. Sodium Thiosulfate is reduced to hydrogen sulfide, and hydrogen sulfide reacts with an iron salt yielding the typical black iron sulfide. Ferric Ammonium Citrate is the hydrogen sulfide (H₂S) indicator. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Yeast Enriched Peptone	10 g
Dextrose.....	1 g
Lactose	10 g
Sucrose.....	10 g
Ferric Ammonium Citrate.....	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Phenol Red	0.025 g
Agar	13.5 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 60 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into tubes and autoclave at 121°C for 15 minutes.
4. After autoclaving, allow medium to solidify in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light, pink-beige.

Prepared Appearance: Prepared medium is bright red to red-orange, clear to trace hazy.

Expected Cultural Response: Cultural response in Triple Sugar Iron Agar at 35°C after 20 - 24 hours incubation.

Microorganism	Response	Reactions			
		Slant	Butt	Gas	H ₂ S
<i>Escherichia coli</i> ATCC® 25922	growth	A	A	+	-
<i>Proteus mirabilis</i> ATCC® 12453	growth	K	A	-	+
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth	K	K	-	-
<i>Salmonella typhimurium</i> ATCC® 14028	growth	K	A	+/-	+
<i>Shigella flexneri</i> ATCC® 12022	growth	K	A	-	-

The organisms listed are the minimum that should be used for quality control testing.

KEY: A, acid, K, alkaline, +, positive, -, negative, +/-, usually negative

Test Procedure

For specific procedures, refer to appropriate references using Triple Sugar Iron Agar.

Results

An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose. An alkaline slant-alkaline butt (red/red) indicates dextrose or lactose were not fermented (non-fermenter). Cracks, splits, or bubbles in medium indicate gas production. A black precipitate in butt indicates hydrogen sulfide production.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Padron and Dockstader⁸ found not all H₂S positive *Salmonella* are positive on TSI.
2. Sucrose is added to TSI to eliminate some sucrose-fermenting non-lactose fermenters, such as *Proteus* and *Citrobacter* spp.⁹
3. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.
4. Do not use inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing butt, mechanical splitting of medium occurs, causing a false positive result for gas production.⁹

Packaging

Triple Sugar Iron Agar	Code No.	7162A	500 g
		7162B	2 kg
		7162C	10 kg

References

1. Russell, F. F. 1911. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J. Med. Res. **25**:217.
2. Kligler, I. J. 1917. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health **7**:1042-1044.
3. Kligler, I. J. 1918. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. J. Exp. Med. **28**:319-322.
4. Sulkin, S. E., and J. C. Willett. 1940. A triple sugar-ferrous sulfate medium for use in identification of enteric organisms. J. Lab. Clin. Med. **25**:649-653.
5. P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
6. Marshall, R. T. (ed.). Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
7. Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, M.D.
8. Padron, A. P. and W. B. Dockstader. 1972. Selective medium for hydrogen sulfide production. Appl. Microbiol. **23**:1107.
9. MacFaddin, J. F. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTIC SOY AGAR (7100)

Intended Use

Tryptic Soy Agar is used for the cultivation of a wide variety of microorganisms. Tryptic Soy Agar conforms with the formula specified in the US Pharmacopeia, USP.¹

Product Summary and Explanation

In 1955, Leavitt et al.² discovered Tryptic Soy Agar (TSA) facilitated vigorous growth of aerobic and anaerobic microorganisms. TSA, a general purpose medium, is commonly referred to as Soybean-Casein Digest Agar USP 23. TSA is a nutritious base, and a variety of supplements can be added to enhance this medium. The addition of 5% sterile, defibrinated sheep, horse, or rabbit blood provides an excellent non-selective medium, used to determine hemolytic reactions of bacteria. TSA supplemented with lecithin and Tween 80® is widely used in environmental monitoring.³

TSA is recommended in multiple water & wastewater applications,⁴ and numerous standard methods for food testing.⁵ Clinically, TSA is used in the differentiation of *Haemophilus* species (the X and V factors are omitted from this medium), and widely used for blood cultures. TSA is commonly used as a maintenance medium for culture collections, and testing bacterial contaminants in cosmetics.⁶

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal provide the nitrogen, vitamins and carbon in TSA. Sodium Chloride maintains osmotic balance in the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein 15 g
Enzymatic Digest of Soybean Meal 5 g
Sodium Chloride 5 g
Agar 15 g
Final pH 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 to 10% blood agar by adding appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 – 50°C .

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and light beige.

Prepared Appearance: Prepared medium without enrichment is clear to slight hazy, yellow beige in color, and free of sediment. Prepared medium with 5% sheep blood is red and opaque.

Expected Cultural Response: Cultural response in TSA at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	growth	--
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using TSA.^{1,3-6}

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C . Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Tryptic Soy Agar	Code No.	7100A	500 g
		7100B	2 kg
		7100C	10 kg

References

1. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
2. **Leavitt, J. M., I. J. Naidorf and P. Shugaevsky.** 1955. The undetected anaerobe in endodontics: a sensitive medium for detection of both aerobes and anaerobes. The NY J. Dentist. **25**:377-382.
3. **Orth, D. S.** 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, NY.
4. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **U.S. Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Curry, A. S., G. G. Joyce, and G. N. McEwen, Jr.** 1993. CTFA Microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Inc. Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTIC SOY AGAR w/ LECITHIN & TWEEN 80 (7163)

Intended Use

Tryptic Soy Agar w/ Lecithin & Tween 80 is used for the isolation of microorganisms from surfaces sanitized with quaternary ammonium compounds.

Product Summary and Explanation

In 1955, Leavitt et al. discovered Tryptic Soy Agar supported excellent growth of aerobic and anaerobic microorganisms.¹ Tryptic Soy Agar is a nutritious base and a variety of supplements are added to enhance the medium, including Lecithin and Tween 80. The Lecithin and Tween 80 inactivate some preservatives that may inhibit bacterial growth, reducing "preservative carryover".² Tryptic Soy Agar w/ Lecithin & Tween 80 is recommended for determining the sanitation efficiency of containers, equipment, and work area (environmental monitoring).

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal provide nitrogen, vitamins, and carbon in Tryptic Soy Agar w/Lecithin & Tween 80. Sodium Chloride maintains osmotic balance in the medium. Lecithin and Tween 80 are added to neutralize surface disinfectants.^{2,3,4} Lecithin is added to neutralize quaternary ammonium compounds. Tween 80 is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol.⁵ Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Lecithin.....	0.7 g
Tween 80	5 g
Agar	20.5 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 51.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, lumpy, and beige.

Prepared Appearance: Prepared medium is trace hazy and beige.

Expected Cultural Response: Cultural response on Tryptic Soy Agar w/ Lecithin & Tween 80 at 35°C after 24 - 48 hours incubation.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 6633	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Clostridium sporogenes</i> ATCC® 11437	growth
<i>Enterococcus faecalis</i> ATCC® 19433	growth
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Salmonella typhimurium</i> ATCC® 14028	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Staphylococcus epidermidis</i> ATCC® 12228	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using Tryptic Soy Agar w/ Lecithin & Tween 80 or environmental monitoring.

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Tryptic Soy Agar w/ Lecithin & Tween 80	Code No.	7163A	500 g
		7163B	2 kg
		7163C	10 kg

References

1. **Leavitt, J. M., I. J. Naidorf and P. Shugaevsky.** 1955. The undetected anaerobe in endodontics: a sensitive medium for detection of both aerobes and anaerobes. The NY J. Dentist. **25**:377-382.
2. **Orth, D. S.** 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, NY.
3. **Quisno, R., I. W. Gibby, and M. J. Foter.** 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118**:320-323.
4. **Erlandson, A. L., Jr., and C. A. Lawrence.** 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science **118**:274-276.
5. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. App. Environ. Microbiol. **32**:80-84.
6. **Favero (chm.).** 1967. Microbiological sampling of surfaces – a state of the art report. Biological Contamination Control Committee, American Association of Contamination Control.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTIC SOY BROTH (7164)

Intended Use

Tryptic Soy Broth is used for the cultivation of a wide variety of microorganisms. Tryptic Soy Broth conforms with the formula specified in the US Pharmacopeia, USP.¹

Product Summary and Explanation

Tryptic Soy Broth, a general purpose medium, is commonly referred to as Soybean-Casein Digest Medium, and abbreviated as TSB. This medium was originally developed for use without blood in determining the effectiveness of sulfonamides against pneumococci and other organisms.² Clostridia and non-sporulating anaerobes grow luxuriantly in this broth when incubated under anaerobic conditions. TSB is recommended for testing bacterial contaminants in cosmetics³ and complies with established standards^{4,5} in the food industry. TSB was chosen by the USDA Animal and Plant Health Inspection Service for detecting viable bacteria in live vaccines.⁶

Tryptic Soy Broth is recommended by the National Committee for Clinical Laboratory Standards (NCCLS)⁷ for inoculum preparation in disk diffusion sensitivity tests. The rich nutritional base of TSB, supplemented with SPS and CO₂, is an excellent broth for blood cultures in clinical applications.⁸ With the addition of 6.5% NaCl, TSB can be used for the selective growth of group D streptococci.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal are nitrogen sources in TSB. Dextrose is the carbon energy source that facilitates organism growth. Sodium Chloride maintains osmotic balance; Dipotassium Phosphate is a buffering agent.

Formula / Liter

Enzymatic Digest of Casein	17g
Enzymatic Digest of Soybean Meal	3 g
Sodium Chloride	5 g
Dipotassium Phosphate	2.5 g
Dextrose.....	2.5 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 30 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear to very light amber.

Expected Cultural Response: Cultural response in TSB at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Bacteroides vulgatus</i> ATCC® 8482	fair to excellent
<i>Escherichia coli</i> ATCC® 25922	good to excellent
<i>Neisseria meningitidis</i> ATCC® 13090	poor to good
<i>Staphylococcus aureus</i> ATCC® 25923	good to excellent
<i>Staphylococcus epidermidis</i> ATCC® 12228	fair to excellent
<i>Streptococcus pneumoniae</i> ATCC® 6305	fair to excellent
<i>Streptococcus pyogenes</i> ATCC® 19615	good to excellent

The organisms listed are the minimum that should be used for quality control testing.

USP Growth Promotion Testing: Cultural response in TSB within 7 days incubation under appropriate temperatures and conditions.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Bacillus subtilis</i> ATCC® 6633	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Micrococcus luteus</i> ATCC® 9341	growth

The organisms listed are the minimum that should be used for USP Growth Promotion testing.

Test Procedure

Refer to appropriate references for specific procedures using Tryptic Soy Broth.^{1,3-8}

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original pale to light beige. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Tryptic Soy Broth	Code No.	7164A	500 g
		7164B	2 kg
		7164C	10 kg

References

1. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
2. **McCullough, N. B.** 1949. Laboratory tests in the diagnosis of brucellosis. Amer. J. of Public Health. **39**:866-869.
3. **Curry, A. S., G. G. Joyce, and G. N. McEwen, Jr.** 1993. CTFA Microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Inc. Washington, D.C.
4. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
5. **Cunniff, P.** 1995. Official methods of analysis AOAC International, 16th ed. AOAC International, Arlington, VA.
6. **Federal Register.** 1992. Detection of viable bacteria and fungi except in live vaccine. Fed. Regist. **21**:113.26.
7. **National Committee for Clinical Laboratory Standards.** 1994. Performance standards for antimicrobial disk susceptibility tests, M2-A5, vol.13, No.24. National Committee for Clinical Laboratory Standards, Villanova, PA.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTONE (7351)

Intended Use

Tryptone is an enzymatic digest of casein for use in preparing microbiological culture media.

Product Summary and Explanation

Tryptone is an enzymatic digest of casein used as a nitrogen source in culture media. Casein is the main protein of milk, and a rich source of amino-acid nitrogen. Tryptone is rich in tryptophane, making it valuable for use in detecting indole production. The absence of detectable levels of carbohydrates in Tryptone makes it a suitable peptone in differentiating bacteria on the basis of their ability to ferment various carbohydrates.

Several media containing Tryptone are specified in standard methods for multiple applications.¹⁻⁴

Principles of the Procedure

Tryptone provides nitrogen, amino acids, and vitamins in microbiological culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and light beige to tan.

Prepared Appearance (2% wt/vol): Prepared medium is clear, yellow with no or a light precipitate.

pH (2% Solution at 25°C): 6.8 - 7.3

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35°C after 18 - 24 incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to excellent growth

Test Procedure

Refer to appropriate references for specific procedures using Tryptone.¹⁻⁴

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Tryptone at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Tryptone should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Tryptone in its intact container when stored as directed.

Packaging

Tryptone	Code No.	7351A	500 g
		7351B	2 kg
		7351C	10 kg

References

1. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8thed., AOAC International, Gaithersburg, MD.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTONE GLUCOSE EXTRACT AGAR (7242)

Intended Use

Tryptone Glucose Extract Agar is used for the cultivation and enumeration of microorganisms in dairy and bottled water products.

Product Summary and Explanation

In the 1930's, Bower and Hucker developed a medium for detecting bacteria in milk and other dairy products.¹ Prickett used a glucose agar containing tryptone to study thermophilic bacteria in milk.² This medium is known as Yeast Dextrose Agar. In 1948, the American Public Health Association (APHA) adopted Tryptone Glucose Extract Agar for use in testing milk and dairy products.³ Currently, APHA specifies Tryptone Glucose Extract Agar for the heterotrophic plate count procedure in testing bottled water.⁴

Tryptone Glucose Extract Agar is also known as Yeast Dextrose Agar.

Principles of the Procedure

Enzymatic Digest of Casein and Beef Extract provides nitrogen, minerals, vitamins, and amino acids in Tryptone Glucose Extract Agar. Dextrose supplies carbon as an energy source. Agar is the solidifying agent.

Formula / Liter

Beef Extract 3 g
Enzymatic Digest of Casein 5 g
Dextrose..... 1 g
Agar 15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 24 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light medium tan.

Prepared Appearance: Prepared medium is trace to slightly hazy, light to medium amber.

Expected Cultural Response: Cultural response on TGE Agar at 35°C after 18 - 24 hours incubation (test strains) or after 47 - 49 hours at 32 ± 1°C for the raw milk sample.

Microorganism	Response
<i>Bacillus subtilis</i> ATCC® 9372	growth
<i>Micrococcus luteus</i> ATCC® 9341	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Sample	Response
Unpasteurized (raw) milk	Excellent growth and recovery w/ a t-value < 2.70

Test Procedures

Pour Plate Method

1. Follow the pour plate method as described in Standard Methods or by laboratory policy.⁴
2. Incubate the inoculated medium in a humid atmosphere at $32 \pm 1^\circ\text{C}$ for 47 - 49 hours incubation for dairy samples or at $35 \pm 0.5^\circ\text{C}$ for at least 72 hours for water samples.

Results

Count total colonies and record results.

Storage

Store sealed bottle containing dehydrated medium at $2 - 30^\circ\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Tryptone Glucose Extract Agar	Code No.	7242A	500 g
		7242B	2 kg
		7242C	10 kg

References

1. **Bowers and Hucker.** 1935. Tech. Bull. 228. NY State Agar. Exp. Sta.
2. **Prickett.** 1928. Tech. Bull. 147. NY State Agar. Exp. Sta.
3. **American Public Health Association.** 1948. Standard methods for the examination of dairy products, 9th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTOSE AGAR (7347)

Intended Use

Tryptose Agar is used for the cultivation of *Brucella* spp. and other fastidious microorganisms.

Product Summary and Explanation

Tryptose Agar is prepared with Tryptose, and 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. Huddleson found Tryptose media to be equal or superior to meat infusion media, providing uniformity for the cultivation and differentiation of fastidious organisms.¹

The high productivity of Tryptose media in the isolation and cultivation of *Brucella* spp. supports the use of this formula as a general purpose medium. Tryptose Agar with 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae.² Tryptose Agar is specified in the Compendium of Methods for the Microbiological Examination of Food.³ Tryptose media are recommended in standard methods for food testing.⁴

Principles of the Procedure

The nitrogen, vitamins, and carbon sources are provided by Tryptose in Tryptose Agar. Dextrose is the carbohydrate source. Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent.

Formula / Liter

Tryptose 20 g
Sodium Chloride 5 g
Dextrose..... 1 g
Agar 15 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 41 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is yellow-beige and trace to slightly hazy.

Expected Cultural Response: Cultural response on Tryptose Agar at 35°C after 48 to 72 hours of incubation.

Microorganism	Response
<i>Brucella abortus</i> ATCC® 4315	growth
<i>Brucella melitensis</i> ATCC® 4309	growth
<i>Brucella suis</i> ATCC® 4314	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the application of Tryptose Agar.

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Tryptose Agar	Code No.	7347A	500 g
		7347B	2 kg
		7347C	10 kg

References

1. **Huddleson, I. F.** 1943. *Brucellosis in man and animals*. Rev. Ed. The Commonwealth Fund, New York.
2. **Moyer, N. P., and L. A. Holcomb.** 1995. *Brucella*. In P.R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. *Compendium of methods for the microbiological examination of food*, 3rd ed. American Public Health Association, Washington, D.C.
4. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodhamel.** 1995. *FDA Bacteriological analytical manual*, 8th ed. AOAC International, Arlington, VA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTOSE BLOOD AGAR BASE (7282)

Intended Use

Tryptose Blood Agar Base is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Investigations of the nutritive properties of tryptose found culture media prepared with this peptone were superior to meat infusion peptone media previously used for the cultivation of *Brucella*, streptococci, pneumococci, and meningococci. Casman reported that a medium consisting of 2% tryptose, 0.3% beef extract, 0.5% sodium chloride, 1.5% agar, and 0.03% dextrose equaled fresh beef infusion base with respect to growth of organisms.^{1,2}

Tryptose Blood Agar Base is a nutritious, infusion-free basal medium typically supplemented with 5 – 10% sheep, rabbit, or horse blood. This medium is typically used for isolating, cultivating, and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, this blood agar base can be used as a general purpose medium. Tryptose Blood Agar Base is specified in standard methods for food testing.³

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Tryptose and Beef Extract in Tryptose Blood Agar Base. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β -hemolytic streptococci.² Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms, and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.⁴

Formula / Liter

Tryptose	10 g
Beef Extract	3 g
Sodium Chloride	5 g
Agar	15 g

Final pH: 7.2 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 33 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light tan.

Prepared Appearance: Prepared medium without blood is yellow-beige, trace to light hazy. With 5% sheep blood the medium is red and opaque.

Expected Cultural Response: Cultural response on Tryptose Blood Agar Base supplemented with and without blood at 35°C after 18 - 24 hours incubation.

Microorganism	Response (Without Sheep Blood)	Reactions (Without Sheep Blood)
<i>Escherichia coli</i> ATCC® 25922	growth	---
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	growth	beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, and 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.⁴
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Results

Examine the medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:⁵

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime-hemolysis (α') is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. 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
2. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.⁴ For optimal performance, incubate blood agar base media under increased CO₂ (5 - 10%) in accordance with established laboratory procedures.

Packaging

Tryptose Blood Agar Base	Code No.	7282A	500 g
		7282B	2 kg
		7282C	10 kg

References

1. **Casman, E. P.** 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. *J. Bacteriol.* **43**:33.
2. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci, and streptococci. *Am. J. Clin. Pathol.* **17**:281-289.
3. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. *FDA Bacteriological analytical manual*, 8th ed. AOAC International, Arlington, VA.
4. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D. C.
5. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, *Clinical microbiology procedures handbook*, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTOSE BROTH (7367)

Intended Use

Tryptose Broth is used for the cultivation of *Brucella* spp. and other fastidious microorganisms.

Product Summary and Explanation

Tryptose Broth is prepared with Tryptose, and 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 studying growth requirements of *Brucella* spp. Huddleson found Tryptose media to be equal or superior to meat infusion media, providing uniformity for the cultivation and differentiation of fastidious organisms.¹

Tryptose Broth can be used a complete basal medium or supplemented with enrichment. Huddleson used a broth containing 2% Tryptose as an enrichment medium in the isolation of *Brucella* spp. from clinical specimens.² McCullough et al. reported that addition of thiamine, dextrose, and iron salts increased growth of *Brucella suis*.³ Addition of 0.1% agar to Tryptose Broth can increase growth of aerobes and anaerobes in liquid media. Tryptose media are recommended in standard methods for food testing.⁴

Principles of the Procedure

The nitrogen, vitamins, and carbon sources are provided by Tryptose in Tryptose Broth. Dextrose is the carbohydrate source. Sodium Chloride maintains the osmotic environment.

Formula / Liter

Tryptose 20 g

Sodium Chloride 5 g

Dextrose..... 1 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 26 g of the medium in one liter of purified water.
2. Heat with frequent agitation to obtain solution.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is light to medium yellow-gold and clear to slightly hazy.

Expected Cultural Response: Cultural response in Tryptose Broth at 35°C after 48 hours incubation.

Microorganism	Response
<i>Brucella abortus</i> ATCC® 4315	growth
<i>Brucella melitensis</i> ATCC® 4309	growth
<i>Brucella suis</i> ATCC® 4314	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the application of Tryptose Broth.

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Tryptose Broth	Code No.	7367A	500 g
		7367B	2 kg
		7367C	10 kg

References

1. **Huddleson, I. F.** 1943. Brucellosis in man and animals. Rev. Ed. The Commonwealth Fund, New York.
2. **Huddleson, I. F.** 1939. Brucellosis in man and animals. 14. Oxford University Press, Oxford, Eng.
3. **McCullough, W. G., R. C. Mills, E. J. Herbst, W. G. Roessler, and C. R. Brewer.** 1947. Studies on the nutritional requirements of *Brucella suis*. J. Bacteriol. **53**:5-15.
4. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

TRYPTOSE PHOSPHATE BROTH (7278)

Intended Use

Tryptose Phosphate Broth is used for the cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Tryptose Phosphate Broth is an infusion-free buffered medium recommended for the cultivation of fastidious, pathogenic microorganisms.

Tryptose Phosphate Broth is valuable in tissue culture procedures, as shown by Ginsberg, Gold, and Jordan. The proteose content of Tryptose Phosphate Broth is considered to be a stimulating factor for cells. Tryptose Phosphate Broth is specified for cell culture procedures.²

Principles of the Procedure

The nitrogen, vitamins, and carbon sources are provided by Enzymatic Digest of Casein. Dextrose is the carbohydrate source. Sodium Chloride maintains the osmotic environment. Disodium Phosphate is the buffering agent.

The addition of 0.1 – 0.2% agar to Tryptose Phosphate Broth facilitates anaerobic growth, and aids in dispersion of reducing substances and CO₂ formed in the environment.³ The low agar concentration provides suitable conditions for aerobic growth in the upper zone, and microaerophilic and anaerobic growth in the lower zone.

Formula / Liter

Enzymatic Digest of Casein 20 g

Dextrose..... 2 g

Sodium Chloride 5 g

Disodium Phosphate..... 2.5 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 29.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light tan.

Prepared Appearance: Prepared medium is gold and clear.

Expected Cultural Response: Cultural response in Tryptose Phosphate Broth at 35°C after 24 - 72 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Staphylococcus aureus</i> ATCC® 25923	growth
<i>Streptococcus pneumoniae</i> ATCC® 6305	growth
<i>Streptococcus pyogenes</i> ATCC® 19615	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for a complete discussion on the application of Tryptose Phosphate Broth.

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Tryptose Phosphate Broth	Code No.	7278A	500 g
		7278B	2 kg
		7278C	10 kg

References

1. **Ginsberg, Gold, and Jordan.** 1955. Proc. Soc. Exp. Biol. Med. **89**:66.
2. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 802-804. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

UNIVERSAL BEER AGAR (7574)

Intended Use

Universal Beer Agar is used for the cultivation of bacteria and yeasts encountered in the brewing industry.

Product Summary and Explanation

Universal Beer Agar was developed by Kozulis and Page, and used as a basal medium supplemented with beer.¹ The developers compared this formula with other media commonly used in breweries for detecting microbial contamination.² The characteristics of Universal Beer Agar are closer to natural environmental conditions found in a typical brewery. Universal Beer Agar supports the growth of more lactic acid bacteria and yields larger colonies in a shorter time than traditional brewer's media. Supplementing this medium with beer has produced a selective environment for organisms that are adapted 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 yeasts that are known beer contaminants. Universal Beer Agar is abbreviated as UBA.

Principles of the Procedure

Tomato Juice Solids is a source of carbon, protein, and nutrients. Yeast Extract provides trace elements, vitamins, and amino acids. Dextrose supplies additional carbon. Dipotassium Phosphate and Monopotassium Phosphate are the buffers. Magnesium Sulfate, Ferrous Sulfate, and Manganese Sulfate are sources of ions that stimulate metabolism. Sodium Chloride maintains the osmotic balance of the medium. Peptonized Milk contains lactose as an energy source. Agar is the solidifying agent.

Formula / Liter

Tomato Juice Solids	7 g
Yeast Extract.....	10 g
Dextrose.....	10 g
Dipotassium Phosphate.....	0.5 g
Monopotassium Phosphate	0.5 g
Magnesium Sulfate	0.125 g
Sodium Chloride	0.01 g
Ferrous Sulfate	0.01 g
Manganese Sulfate	0.01 g
Peptonized Milk.....	15 g
Agar	12 g

Final pH: 6.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 55 g of the medium in 750 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. While medium is hot, add and mix 250 mL of beer without degassing.
4. Autoclave at 121°C for 10 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and amber.

Expected Cultural Response: Cultural response on Universal Beer Agar at 30°C after 18 - 72 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Lactobacillus casei</i> ATCC® 393	growth
<i>Lactobacillus fermentum</i> ATCC® 9338	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Universal Beer Agar	Code No.	7574A	500 g
		7574B	2 kg
		7574C	10 kg

References

1. **Kozulis, J. A., and H. E. Page.** 1968. A new universal beer agar medium for the enumeration of wort and beer microorganisms. Proc. Am. Soc. Brew. Chem. **19**:52-58.
2. **Murphy, D. T., and L. T. Saletan.** 1970. Use of microbiological media in the brewery. Tech. Q. Master Brew. Assoc. Am. **7**:182-187.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1. p. 819-820. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

UNIVERSAL PRE-ENRICHMENT BROTH (7510)

Intended Use

Universal Pre-Enrichment Broth is used for the recovery of *Salmonella* spp. and *Listeria* spp.

Product Summary and Explanation

Traditional methods for recovering *Salmonella* and *Listeria* from food products require separate pre-enrichment media for each microorganism.^{1,2} Some broth media recommended for pre-enrichment contain antibiotic inhibitors,³ or have insufficient buffering capacity which hinder recovery of sublethally injured cells.³⁻⁵

Bailey and Cox³ formulated Universal Pre-Enrichment 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

Enzymatic Digest of Casein and Proteose Peptone are the nitrogen sources in Universal Pre-Enrichment Broth. Sodium and Potassium Phosphates buffer the medium. Sodium Chloride, Magnesium Sulfate and Ferric Ammonium Citrate provide essential ions. Dextrose is an energy source, and Sodium Pyruvate helps stimulate the metabolism of stressed organisms.

Formula / Liter

Enzymatic Digest of Casein	5 g
Proteose Peptone	5 g
Potassium Phosphate Monobasic	15 g
Sodium Phosphate Dibasic	7 g
Sodium Chloride	5 g
Dextrose.....	0.5 g
Magnesium Sulfate	0.25 g
Ferric Ammonium Citrate.....	0.1 g
Sodium Pyruvate.....	0.2 g

Final pH: 6.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 38 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium, if necessary.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is light to medium amber and clear.

Expected Cultural Response: Cultural response in Universal Pre-Enrichment Broth at 30°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Listeria monocytogenes</i> ATCC® 7644	good growth
<i>Listeria monocytogenes</i> ATCC® 15313	good growth
<i>Salmonella enteritidis</i> ATCC® 13076	good growth
<i>Salmonella typhimurium</i> ATCC® 14028	good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Substitute Universal Pre-Enrichment Broth for pre-enrichment media as specified for *Salmonella* and *Listeria*^{1,2} and follow recommended procedures.

Results

Salmonella and *Listeria* demonstrate good growth and recovery following pre-enrichment in this broth.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Universal Pre-Enrichment Broth	Code No.	7510A	500 g
		7510B	2 kg
		7510C	10 kg

References

1. **Vanderzant, C., and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
3. **Bailey, J. S., and N. A. Cox.** 1992. Universal preenrichment broth for the simultaneous detection of *Salmonella* and *Listeria* in foods. J. Food Protect **55**:256-259.
4. **Bailey, J. S., D. L. Fletcher, and N. A. Cox.** 1990. Efficacy of enrichment media for recovery of heat-injured *Listeria monocytogenes*. J. Food Prot. **47**:299-302.
5. **Juven, B. J., N. A. Cox, J. S. Bailey, J. E. Thomson, O. W. Charles, and J. V. Shutze.** 1984. Recovery of *Salmonella* from artificially contaminated poultry feed in non-selective and selective broth media. J. Food Prot. **47**:299-302.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

UREA AGAR BASE (7226)

Intended Use

Urea Agar Base is used for the differentiation of microorganisms on the basis of urease production.

Product Summary and Explanation

Christensen devised a urea agar medium containing peptone and dextrose that had a reduced buffer content.¹ The medium supported a vigorous growth of many gram-negative, enteric bacilli and readily permitted observation of urease production. Ewing used Urea Agar Base as a differential medium in the examination of many cultures from stool specimens.² Urea Agar Base may be used as a screening medium (along with Triple Sugar Iron Agar) for the selection of *Salmonella* and *Shigella* cultures for serologic classification.³ Urea Agar Base is used to detect production of urease by yeast.⁴ Urease production is an important differential test in microbiology and outlined in standard methods.⁵⁻⁷

Principles of the Procedure

Enzymatic Digest of Gelatin provides nitrogen, carbon, and amino acids required for organism growth in Urea Agar Base. Dextrose is an energy source. Sodium Chloride maintains the osmotic balance of the medium. Monopotassium Phosphate is the buffer. Urea provides a nitrogen source for organisms producing urease. The splitting of urea by urease causes the release of ammonia, increasing pH of the medium to the alkaline side. This is indicated by a color change of the pH indicator, Phenol Red, from yellow (pH 6.8) to red (pH 8.1). Agar is added as a supplement to solidify the medium.

Formula / Liter

Enzymatic Digest of Gelatin	1 g
Dextrose.....	1 g
Sodium Chloride	5 g
Monopotassium Phosphate	2 g
Urea	20 g
Phenol Red	0.012 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Agar, Bacteriological 15 g

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. Harmful by inhalation, ingestion, and through skin absorption. Irritant to skin, mucous membranes, and eyes.

Directions

1. Suspend 29 g of the medium in 100 mL of purified water until dissolved completely. Filter sterilize.
2. Suspend 15 g of agar in 900 mL of purified water.
3. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
4. Autoclave at 121°C for 15 minutes.
5. Cool sterilized agar to 45 - 50°C and aseptically add the sterile Urea Agar Base.
6. Mix thoroughly and dispense into sterile tubes. Place tubes in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and off-white.

Prepared Appearance: Prepared medium is light to medium yellow-orange and trace to slight hazy.

Expected Cultural Response: Cultural response on Urea Agar Base supplemented with agar at 35°C after 18 - 24 hours incubation.

Microorganism	Reactions
<i>Escherichia coli</i> ATCC® 25922	negative
<i>Klebsiella pneumoniae</i> ATCC® 13883	weak positive
<i>Proteus vulgaris</i> ATCC® 13315	positive
<i>Salmonella typhimurium</i> ATCC® 14028	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Use a heavy inoculum from a pure 18 – 24 hour culture. Inoculate by streaking back and forth over the entire slant surface. Do not stab the butt because it serves as a color control.
2. Incubate tubes with loose caps at $35 \pm 2^{\circ}\text{C}$.
3. Observe reactions after 6 and 24 hours, and for the next 6 days.¹ Longer periods of incubation may be necessary.

Results

The production of urease is a positive reaction, indicated by an intense red or pink color on the slant.

No color change of the medium is a negative reaction.

Storage

Store sealed bottle containing the dehydrated medium at $2 - 8^{\circ}\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

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 or other proteins that 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. Do not autoclave medium because excessive heat may alter ingredients.⁸
2. Do not heat or reheat the medium because urea decomposes very easily.
3. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Urea Agar Base detects rapid urease activity of only the urease-positive *Proteus* spp.

Packaging

Urea Agar Base	Code No.	7226A	500 g
		7226B	2 kg
		7226C	10 kg

References

1. Christensen, W. B. 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. J. Bacteriol. **52**:461.
2. Ewing, W. H. 1946. An additional *Shigella paradyseae* serotype. J. Bacteriol. **51**:433-445.
3. Ewing, W. H., and D. W. Bruner. 1947. Selection of *Salmonella* and *Shigella* cultures for serological classification. Am. J. Clin. Pathol. **17**:1-12.
4. Baron, E. J., L. R. Peterson, and S. M. Finegold. 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana. FDA Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. Marshall, R. T. (ed.). 1993. Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
8. MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Technical Information

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UVM MODIFIED LISTERIA ENRICHMENT BROTH (7409)

Intended Use

UVM Modified Listeria Enrichment Broth is used for the selective enrichment of *Listeria* spp.

Product Summary and Explanation

Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann,¹ is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant woman.² Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission include turkey frankfurters,⁴ coleslaw, pasteurized milk, Mexican style cheese, and pate'. *Listeria* spp. are ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, and river water.⁵

UVM Modified Listeria Enrichment Broth is a modification of the formula described by Donnelly and Baigent.⁶ This formula is used for the selective enrichment of *Listeria* spp. from food^{7,8} and clinical specimens.⁹ *Listeria* spp. grow over a pH range of 5.0 - 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, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract provides nitrogen, vitamins, and minerals in UVM Modified Listeria Enrichment Broth. The Phosphates are the buffering agents, Sodium Chloride maintains osmotic balance. Nalidixic Acid inhibits growth of gram-negative organisms. Acriflavin inhibits Gram-positive bacteria. Esculin is hydrolyzed by *Listeria* spp. 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.¹⁰ The high salt tolerance of *Listeria* is used as a means to inhibit growth of enterococci.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue.....	5 g
Beef Extract	5 g
Yeast Extract.....	5 g
Sodium Chloride	20 g
Disodium Phosphate.....	9.6 g
Monopotassium Phosphate	1.35 g
Esculin	1 g
Acriflavin	0.012 g
Nalidixic Acid.....	0.02 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light tan.

Prepared Appearance: Prepared medium is green-yellow to yellow with faint blue-green ring at the surface, and very slightly opalescent.

Expected Cultural Response: Cultural response in UVM Modified Listeria Enrichment Broth at 30°C after 24 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	good growth
<i>Listeria monocytogenes</i> ATCC® 15313	good growth
<i>Staphylococcus aureus</i> ATCC® 25923	suppressed at 18 – 24 hours

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

The U.S.D.A.⁸ method 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 Refer to appropriate references for further information on testing food samples or clinical specimens.⁷⁻⁹

Results

Refer to appropriate references and procedures for results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the color has changed from the original light tan. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. *Listeria* spp., other than *Listeria monocytogenes*, can grow on isolation media. An identification of *Listeria monocytogenes* must be confirmed through biochemical and serological testing.¹⁰

Packaging

UVM Modified Listeria Enrichment Broth	Code No.	7409A	500 g
		7409B	2 kg
		7409C	10 kg

References

1. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bact. **29**:407-439.
2. Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen refrigerated ground beef. J. Food Prot. **57**:969-974.
3. Bremer, P. J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
4. Grau, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
5. Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
6. Donnelly, C. W., and G. J. Baigent. 1986. Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl. Environ. Microbiol. **52**:689-695.
7. Vanderzant, C., and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. Lee, W. H., and D. McClain. 1994. Laboratory Communication No. 57, U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
9. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
10. Fraser, J., and W. Sperber. 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. **51**:762-765.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

VIOLET RED BILE AGAR (7165)

Intended Use

Violet Red Bile Agar is used for the enumeration of coliforms in food and dairy product conforms to American Public Health Association (APHA).

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative anaerobic, gram-negative, non-sporeforming bacilli. Coliforms ferment lactose and form acid and gas at 35°C within 48 hours. Members of *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, (VRBA). If typical coliform colonies appear, they are tested further to confirm their identification as coliforms. Coliform colonies lower the pH of the medium, subsequently causing their colonies to look red (Neutral Red Dye) and to precipitate the bile salts.

Principles of the Procedure

Enzymatic Digest of Gelatin is the source of carbon, nitrogen, vitamins, and minerals in Violet Red Bile Agar. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Bile Salts Mixture and Crystal Violet inhibit most Gram-positive bacteria. Lactose is a carbohydrate source. Neutral Red is a pH indicator. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Enzymatic Digest of Gelatin	7 g
Bile Salts Mixture	1.5 g
Lactose	10 g
Sodium Chloride	5 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.002 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 41.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for two minutes. DO NOT AUTOCLAVE.
3. Cool the medium to 45 - 46°C and dispense 15 - 20 mL into 100 mm petri dishes containing inoculum.
4. After solidification of the inoculated medium, evenly add a cover layer of 4 mL of the cooled (45 -46°C) agar medium.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and red-beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and red-purple.

Expected Cultural Response: Cultural response on Violet Red Bile Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction
<i>Enterobacter aerogenes</i> ATCC® 13048	good growth	pink colonies
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	----
<i>Escherichia coli</i> ATCC® 25922	good growth	pink to red colonies w/ bile ppt
<i>Salmonella typhimurium</i> ATCC® 14028	good growth	colorless colonies

The organisms listed are the minimum that should be used for quality control testing.

Test 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 precipitate bile acids. Continue with confirmatory testing of typical organisms colonies.¹⁻³

Results

Lactose fermenters are purple-red, with or without a zone of precipitate around the colonies. Lactose non-fermenters are colorless to transparent colonies. Gram-positive cocci are colorless, pinpoint colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. VRBA may not be completely inhibitory to Gram-positive organisms, and will grow Gram-negative bacilli other than members of *Enterobacteriaceae*. Perform a Gram stain and biochemical tests to identify isolates.
3. Boiling the medium for longer than 2 minutes can decrease the ability to support growth.

Packaging

Violet Red Bile Agar	Code No.	7165A	500 g
		7165B	2 kg
		7165C	10 kg

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

VIOLET RED BILE AGAR W/ MUG (7359)

Intended Use

Violet Red Bile Agar w/ MUG is used for the detection of coliforms and the fluorogenic detection of *Escherichia coli*.

Product Summary and Explanation

Violet Red Bile Agar w/ MUG is specified in many 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 specify Violet Red Bile Agar with MUG for detecting *E. coli* in food and dairy products by fluorescence.¹⁻³ This medium is often abbreviated as VRBA w/ MUG.

Principles of the Procedure

Enzymatic Digest of Gelatin is the source of carbon, nitrogen, vitamins, and minerals in Violet Red Bile Agar w/ MUG. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Bile Salts Mixture 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.

Formula / Liter

Enzymatic Digest of Gelatin	7 g
Yeast Extract.....	3 g
Bile Salts Mixture	1.5 g
Lactose	10 g
Sodium Chloride	5 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.002 g
4-Methylumbelliferyl- β -D-Glucuronide	0.05 g
Agar	15 g

Final pH: 7.4 $\pm$ 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 41.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for only two minutes to completely dissolve the medium.
3. Cool the medium to 45 - 46°C and pour plates.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and red-beige.

Prepared Appearance: Prepared medium is clear to slightly hazy and red-purple.

Expected Cultural Response: Cultural response on Violet Red Bile Agar w/ MUG at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions	
		Colonies	Fluorescence
<i>Enterobacter aerogenes</i> ATCC® 13048	good growth	pink colonies	negative
<i>Escherichia coli</i> ATCC® 25922	good growth	pink-red colonies w/ bile ppt	positive
<i>Salmonella typhimurium</i> ATCC® 14028	good growth	colorless colonies	negative
<i>Staphylococcus aureus</i> ATCC® 25923	inhibited	-----	negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate.¹⁻³
2. Incubate plates at 35°C for 22 - 26 hours.
3. Examine plates for growth and fluorescence.

Results

Coliform organisms form purple-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.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. 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 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.

Packaging

Violet Red Bile Agar w/ MUG	Code No.	7359A	500 g
		7359B	2 kg
		7359C	10 kg

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Vanderzant, C. and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
4. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl Environ. Microbiol. **43**:1320-1329.
5. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of β -D-glucuronidase negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.
6. **Hansen, W., and E. Yourassowsky.** 1984. Detection of β -D-glucuronidase in lactose fermenting members of the family *Enterobacteriaceae* and its presence in bacterial urine cultures. J. Clin. Microbiol. **20**:1177-1179.
7. **Kilian, M., and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. Acta. Pathol. Microbiol. Scand. Sect. B, **84**:245-251.
8. **Mates, A., and M. Shaffer.** 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriol. **67**:343-346.
9. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. J. of Food Sci. **50**:1736-1746.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

VIOLET RED BILE GLUCOSE AGAR (7425)

Intended Use

Violet Red Bile Glucose Agar is used for the enumeration of *Enterobacteriaceae* in foods.

Product Summary and Explanation

The *Enterobacteriaceae* group includes lactose-fermenting coliform bacteria, nonlactose-fermenting strains of *E. coli*, and nonlactose-fermenting species such as *Salmonella* and *Shigella*. When examining certain foods, it is important to detect *Enterobacteriaceae* rather than coliform bacteria.^{1,2} *Enterobacteriaceae* are glucose-fermenting bacteria. Mossel et al.³ modified Violet Red Bile Agar, which contains lactose, by adding glucose to improve recovery of *Enterobacteriaceae*. Further research by Mossel et al.^{4,5} demonstrated that lactose could be omitted, resulting in the formulation known as Violet Red Bile Glucose Agar.

Principles of the Procedure

Enzymatic Digest of Gelatin provides nitrogen, amino acids and carbon in Violet Red Bile Glucose Agar. Yeast Extract supplies essential vitamins for organism growth. Dextrose is the carbohydrate. Bile Salts and Crystal Violet are selective agents, inhibiting gram-positive cocci and allowing gram-negative organisms to grow. Dextrose fermenters produce red colonies with red-purple halos in the presence of Neutral Red, the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	7 g
Yeast Extract.....	3 g
Dextrose.....	10 g
Bile Salts	1.5 g
Sodium Chloride	5 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.002 g
Agar	13.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system and skin.

Directions

1. Suspend 40 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.
4. Cool to 45 - 50°C and dispense into sterile petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pink-beige.

Prepared Appearance: Prepared medium is red-purple and trace to slightly hazy.

Expected Cultural Response: Cultural response on Violet Red Bile Glucose Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterobacter aerogenes</i> ATCC® 13048	growth	pink colonies with reddish precipitate
<i>Escherichia coli</i> ATCC® 25922	growth	pink colonies with reddish precipitate
<i>Salmonella typhimurium</i> ATCC® 14028	growth	pink colonies with reddish precipitate
<i>Staphylococcus aureus</i> ATCC® 25923	growth	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Violet Red Bile Glucose Agar is 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 prevent swarming colonies and provide semi-anaerobic conditions suppressing growth of nonfermentative gram-negative organisms. Stab inoculation procedures can also be used with Violet Red Bile Glucose Agar.

Results

Enterobacteriaceae ferment dextrose, produce acid products, and form pink to reddish colonies with reddish precipitate.

Storage

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. When used in the pour plate procedure, Violet Red Bile Glucose Agar should be freshly prepared, tempered to 47°C, and used within 3 hours.

Packaging

Violet Red Bile Glucose Agar	Code No.	7425A	500 g
		7425B	2 kg
		7425C	10 kg

References

1. **Draft Standard Methods for Microbiological Examination of Meat Products.** 1977. Part 3: Detection and enumeration of *Enterobacteriaceae*. BS5393: Part 3, ISO/DIS 5552.
2. **Mossel, D. A. A.** 1985. Media for *Enterobacteriaceae*. Int. J. Food Microbiol. **2**:27.
3. **Mossel, D. A. A., W. H. J. Mengerink, and H. H. Scholts.** 1962. Use of a modified MacConkey agar medium for the selective growth and enumeration of *Enterobacteriaceae*. J. Bacteriol. **84**:381.
4. **Mossel, D. A. A., I. Eelderink, M. Koopmans, and F. van Rossem.** 1978. Lab Practice. **27**:1049-1050.
5. **Mossel, D. A. A., I. Eelderink, M. Koopmans, and F. van Rossem.** 1979. Influence of carbon source, bile salts and incubation temperature on recovery of *Enterobacteriaceae* from food using MacConkey-type agars. J. Food Protect. **42**:470-475.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

VOGEL AND JOHNSON AGAR (7207)

Intended Use

Vogel and Johnson Agar is used for the isolation of staphylococci.

Product Summary and Explanation

Coagulase-positive staphylococci, primarily *Staphylococcus aureus*, are among the microorganisms that cause spoilage or chemical changes in cosmetic products.¹ To isolate coagulase-positive, mannitol fermenting staphylococci, Vogel and Johnson² modified Tellurite-Glycine Agar by Zebovitz, Evans, and Niven.³ The modification included increasing mannitol and adding a pH indicator. Vogel and Johnson Agar selects and differentiates coagulase-positive staphylococci that ferment mannitol and reduce tellurite.⁴

Vogel and Johnson (VJ) Agar is specified in standard methods testing for cosmetics,^{1,5} pharmaceutical articles,⁶ and nutritional supplements.⁷

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, amino acids, and minerals in Vogel and Johnson Agar. Yeast Extract is a vitamin source to stimulate bacterial growth. Mannitol is the fermentable carbohydrate. Dipotassium Phosphate is the buffering agent. Lithium Chloride, Glycine, and 1% Potassium Tellurite Solution inhibit the growth of most microorganisms except staphylococci. Phenol Red is the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Yeast Extract.....	5 g
Mannitol	10 g
Dipotassium Phosphate.....	5 g
Lithium Chloride	5 g
Glycine	10 g
Phenol Red	0.025 g
Agar	15 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

1% Potassium Tellurite Solution

Precautions

1. For In Vitro Diagnostic Use.
2. TOXIC. Harmful if swallowed, inhaled, or absorbed through skin.

Directions

1. Suspend 60 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. After cooling to 45 - 50°C add 20 mL of a sterile 1% Potassium Tellurite Solution.
5. Mix thoroughly before dispensing.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light red-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and red-orange.

Expected Cultural Response: Cultural response on Vogel and Johnson Agar at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	part. to full inhibited	black colonies
<i>Escherichia coli</i> ATCC® 25923	inhibited	---
<i>Staphylococcus aureus</i> ATCC® 25923	growth	black colonies with yellow zones
<i>Staphylococcus epidermidis</i> ATCC® 12228	partially inhibited	colorless to black colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for the isolation and identification of staphylococci.

Results

Coagulase-positive strains of *S. aureus* reduce tellurite and form black colonies on the medium. These strains typically ferment mannitol and exhibit yellow halos around black colonies. Most organisms other than coagulase - positive staphylococci are inhibited during the first 24 hours of incubation. After 24 hours, other organisms, especially fecal streptococci and coagulase - negative *S. epidermidis* may grow.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Vogel and Johnson Agar	Code No.	7207A	500 g
		7207B	2 kg
		7207C	10 kg

References

1. Hitchins, A. D., T. T. Tran, and J. E. McCarron. 1995. Microbiological methods for cosmetics, p. 23.01-23.11. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
2. Vogel, T. A., and M. Johnson. 1960. A modification of the Tellurite-Glycine Medium for use in the identification of *Staphylococcus aureus*. Public Health Lab. **18**:131.
3. Zebovitz, E., J. B. Evans, and C. F. Niven, Jr. 1955. Tellurite-Glycine Agar; a selective plating medium for the quantitative detection of coagulase-positive staphylococci. J. Bacteriol. **70**:686.
4. MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1, p. 846-849. Williams & Wilkins, Baltimore, MD.
5. Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (eds.). 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
6. United States Pharmacopeial Convention. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

WILKINS-CHALGREN AGAR (7232)

Intended Use

Wilkins-Chalgren Agar is used for the cultivation of anaerobic microorganisms.

Product Summary and Explanation

Wilkins-Chalgren Agar was designed by Wilkins and Chalgren for use in determining minimal inhibitory concentration (MIC's) of antibiotics for anaerobic bacteria by the agar dilution procedure.¹ This medium was selected because it does not require the addition of blood to support satisfactory growth of most anaerobes. Anaerobic bacteria cause a variety of human infections including endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.^{2,3} The survival of anaerobic bacteria is dependent on their sensitivity to oxygen,⁴ nutritional requirements, appropriate collection, culture medium, and incubation time and temperature.⁴

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Gelatin provide nitrogen, amino acids, and minerals in Wilkins-Chalgren Agar. Yeast Extract is added to provide vitamins and other growth factors, including purines and pyrimidines. Sodium Chloride maintains the osmotic balance of the medium. Dextrose is a carbon source. L-Arginine contributes to growth of *Eubacterium lentum*. Sodium Pyruvate provides an energy source for *Veillonella* spp. and degrades hydrogen peroxide. Hemin (X Factor) and Vitamin K are essential for growth of a number of species of anaerobes. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Gelatin	10 g
Yeast Extract.....	5 g
Sodium Chloride	5 g
Dextrose.....	1 g
L-Arginine.....	1 g
Sodium Pyruvate.....	1 g
Hemin.....	0.005 g
Vitamin K.....	0.0005 g
Agar	15 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 48 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is yellow-beige and trace to slightly hazy.

Expected Cultural Response: Cultural response on Wilkins-Chalgren Agar at 35°C after 48 - 72 hours incubation.

Microorganism	Response
<i>Bacteroides fragilis</i> ATCC® 25285	growth
<i>Clostridium novyi</i> ATCC® 7659	growth
<i>Clostridium perfringens</i> ATCC® 13124	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion of aerobic and anaerobic bacteria from clinical specimens, refer to appropriate procedures outlined in the references.⁴⁻⁶ Refer to standard methods for the examination of bacteria in food.^{7,8}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Wilkins-Chalgren Agar	Code No.	7232A	500 g
		7232B	2 kg
		7232C	10 kg

References

1. **Wilkins, T. D., and S. Chalgren.** 1976. Medium for use in antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents. Chemother. **10**:926.
2. **Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadmony (eds.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
3. **Smith, L. D. S.** 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
8. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

WILKINS-CHALGREN BROTH (7233)

Intended Use

Wilkins-Chalgren Broth is used for the cultivation of anaerobic microorganisms.

Product Summary and Explanation

Wilkins-Chalgren Broth is derived from Wilkins-Chalgren Agar.¹ This broth can be used for determining susceptibility of anaerobes to antibiotics. In addition, Wilkins-Chalgren Broth provides good growth of anaerobes. Wilkins and Chalgren formulated Wilkins-Chalgren Agar for use in determining the minimal inhibitory concentration (MIC's) of antibiotics for anaerobic bacteria by the agar dilution procedure.¹ This medium was selected because it does not require the addition of blood to support satisfactory growth of most anaerobes. Wilkins-Chalgren Broth is the same formula as Wilkins-Chalgren Agar with agar omitted.

Anaerobic bacteria cause a variety of human infections including endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.^{2,3} The survival of anaerobic bacteria is dependent on their sensitivity to oxygen, nutritional requirements, appropriate collection, culture medium, and incubation time and temperature.⁴

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Gelatin provide nitrogen, amino acids, and minerals in Wilkins-Chalgren Broth. Yeast Extract supplies essential vitamins and other growth factors, including purines and pyrimidines. Sodium Chloride maintains the osmotic balance of the medium. Dextrose is a carbon source. L-Arginine contributes to growth of *Eubacterium lentum*. Sodium Pyruvate provides an energy source for *Veillonella* spp. and acts as an oxygen scavenger. Hemin (X Factor) and Vitamin K are added as essential growth factors for a number of species of anaerobes.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Gelatin	10 g
Yeast Extract.....	5 g
Sodium Chloride	5 g
Dextrose.....	1 g
L-Arginine.....	1 g
Sodium Pyruvate.....	1 g
Hemin.....	0.005 g
Vitamin K.....	0.0005 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 33 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium amber and brilliantly clear to very slightly hazy.

Expected Cultural Response: Cultural response in Wilkins-Chalgren Broth at 35°C after 24 - 72 hours incubation.

Microorganism	Response
<i>Bacteroides fragilis</i> ATCC® 25285	growth
<i>Clostridium novyi</i> ATCC® 7659	growth
<i>Clostridium perfringens</i> ATCC® 13124	growth
<i>Prevotella melaninogenica</i> ATCC® 25845	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion of aerobic and anaerobic bacteria from clinical specimens, refer to appropriate procedures outlined in the references.⁴⁻⁶ Refer to standard methods for the examination of bacteria in food.^{7,8}

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Wilkins-Chalgren Broth	Code No.	7233A	500 g
		7233B	2 kg
		7233C	10 kg

References

1. **Wilkins, T. D., and S. Chalgren.** 1976. Medium for use in antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents. Chemother. **10**:926.
2. **Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadmony (eds.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
3. **Smith, L. D. S.** 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
8. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Technical Information

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W-L NUTRIENT MEDIUM (7488)

Intended Use

W-L Nutrient Medium is used for the cultivation of yeasts, molds, and bacteria encountered in brewing and industrial fermentations.

Product Summary and Explanation

W-L Nutrient Medium was developed by Green and Gray^{1,2} while studying various fermentation processes. An exhaustive study examining methods of fermentation control procedures in worts, beers, liquid yeasts and similar fermentation products led to the development of W-L Nutrient Medium. At a pH of 5.5, counts of viable baker's yeast will grow on W-L Nutrient Medium.

W-L Nutrient Medium is also referred to as "Wallerstein Laboratory Medium".

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins, and amino acids. Enzymatic Digest of Casein provides nitrogen, amino acids, and carbon. Dextrose is a source of carbohydrate. Monopotassium Phosphate buffers the medium. 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.

Formula / Liter

Yeast Extract.....	4 g
Enzymatic Digest of Casein	5 g
Dextrose.....	50 g
Monopotassium Phosphate	0.55 g
Potassium Chloride.....	0.425 g
Calcium Chloride.....	0.125 g
Magnesium Sulfate	0.125 g
Ferric Chloride	0.0025 g
Manganese Sulfate	0.0025 g
Bromcresol Green.....	0.022 g
Agar	20 g

Final pH: 5.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 80 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige with a green tint.

Prepared Appearance: Prepared medium is trace to slightly hazy and slightly opalescent blue-green.

Expected Cultural Response: Cultural response on W-L Nutrient Medium at 35°C after 18 - 48 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC® 25922	growth
<i>Lactobacillus fermentum</i> ATCC® 9338	growth
<i>Proteus mirabilis</i> ATCC® 12453	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures. For a complete discussion on the isolation and identification of yeasts, refer to references outlined in the references.^{3,4}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

W-L Nutrient Medium	Code No.	7488A	500 g
		7488B	2 kg
		7488C	10 kg

References

1. **Green, S. R., and P. P. Gray.** 1950. Paper read at American Society of Brewing Chemists Meeting. Wallerstein Lab. Commun. 12:43.
2. **Green, S. R., and P. P. Gray.** 1950. A differential procedure applicable to bacteriological investigation in brewing. Wallerstein Lab. Commun. 13:357.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
4. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.6.7. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

XL AGAR BASE (7223)

Intended Use

XL Agar Base is used for the isolation and differentiation of gram-negative enteric bacilli.

Product Summary and Explanation

XL (Xylose Lysine) Agar Base was developed by Taylor¹ for isolating and differentiating gram-negative enteric bacilli. This medium is nonselective, allowing growth of most enteric bacteria. XL Agar is recommended for performing counts of enteric organisms.

XL Agar Base can be supplemented with sodium thiosulfate, ferric ammonium citrate, and sodium deoxycholate to develop a more selective medium, XLD Agar. XL Agar Base can be made selective for *Salmonella* by adding brilliant green (1.25 mL of a 1% aqueous solution per liter) prior to autoclaving. The resulting XLBG Agar inhibits coliforms and *Shigella* spp., and is recommended for isolating *Salmonella* spp. following selenite or tetrathionate enrichment in food analysis.¹

Principles of the Procedure

Yeast Extract provides sources of nitrogen, carbon, and vitamins required for organism growth. Xylose, Lactose, and Sucrose, provide sources of fermentable carbohydrate. Xylose is fermented by most enteric organisms except *Shigella* spp. and *Providencia* spp. Lysine is added to differentiate *Salmonella* spp. As Xylose is exhausted, *Salmonella* organisms decarboxylate lysine causing a reversion to alkaline conditions. Alkaline reversion by other lysine-positive organisms is prevented by excess acid production from fermentation of lactose and sucrose. Acidic conditions inhibit the reaction. Phenol Red is a pH indicator. Sodium Chloride maintains the osmotic balance in the medium. Agar is the solidifying agent.

Formula / Liter

Xylose	3.5 g
L-Lysine.....	5 g
Lactose	7.5 g
Sucrose.....	7.5 g
Sodium Chloride	5 g
Yeast Extract.....	3 g
Phenol Red	0.08 g
Agar	13.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Sodium Thiosulfate, 34%
Ferric Ammonium Citrate, 4%

Precautions

1. For In Vitro Diagnostic Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 45 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50 - 55°C and aseptically add 20 mL of a sterile aqueous solution of 34% sodium thiosulfate and 4% ferric ammonium citrate.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light pink-beige.

Prepared Appearance: Prepared medium is red-orange to bright red and trace to slightly hazy.

Expected Cultural Response: Cultural response on XL Agar Base at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	partial inhibition	yellow colonies
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	yellow colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	red colonies w/ black centers
<i>Shigella sonnei</i> ATCC® 25931	growth	pink colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Stool specimens or rectal swabs may be plated directly. Selective enrichment broths, such as Selenite Broth or Tetrathionate Broth, may be use prior to streaking.²⁻⁴ For specific procedures refer to appropriate references.

Results

Fermentation of xylose, lactose, and sucrose generates acid products, causing a color change in the medium from red to yellow. Phenol Red is used as the acid-base indicator, producing yellow colonies from an acid reaction.

Lysine decarboxylation, in the absence of lactose and sucrose fermentation, results in a reversion to an alkaline condition. This alkaline condition causes the color of the medium to change back to red.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Red, false-positive colonies may occur with *Proteus* spp. and *Pseudomonas* spp.
3. Incubation in excess of 48 hours may lead to false-positive results.

Packaging

XL Agar Base	Code No.	7223A	500 g
		7223B	2 kg
		7223C	10 kg

References

1. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars: new media for isolation of enteric pathogens. Am. J. Clin. Pathol. 44(4):471-475.
2. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
3. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.
4. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. he United States Pharmacopeial Convention, Rockville, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

XLD AGAR (7166)

Intended Use

XLD Agar is used for the isolation and differentiation of enteric pathogens.

Product Summary and Explanation

XL (Xylose Lysine) Agar Base was developed by Taylor¹ for isolating and differentiating gram-negative enteric bacilli. XL Agar Base was supplemented with sodium thiosulfate, ferric ammonium citrate, and sodium deoxycholate to develop a more selective medium, XLD Agar.

XLD Agar was developed principally for isolating *Shigella* spp. and *Providencia* spp., and shown to be an effective differential media.^{2,4}

Principles of the Procedure

Yeast Extract provides sources of nitrogen, carbon, and vitamins required for organism growth. Xylose, Lactose, and Sucrose, provide sources of fermentable carbohydrate. Xylose is fermented by most enteric organisms except *Shigella* spp. and *Providencia* spp. Lysine is added to differentiate *Salmonella*. As Xylose is exhausted, *Salmonella* spp organisms decarboxylate lysine causing a reversion to alkaline conditions. Alkaline reversion by other lysine-positive organisms is prevented by excess acid production from fermentation of lactose and sucrose.

Sodium Thiosulfate and Ferric Ammonium Citrate act as selective agents, allowing visualization of hydrogen sulfide production under alkaline conditions. Sodium Deoxycholate is also a selective agent. Phenol Red is an indicator. Sodium Chloride maintains the osmotic balance in the medium. Agar is the solidifying agent.

Formula / Liter

Yeast Extract.....	3 g
Lactose	7.5 g
Sucrose.....	7.5 g
Xylose	3.5 g
L-Lysine.....	5 g
Ferric Ammonium Citrate.....	0.8 g
Phenol Red	0.08 g
Sodium Chloride	5 g
Sodium Deoxycholate	2.5 g
Sodium Thiosulfate	6.8 g
Agar	13.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. May be harmful if swallowed or inhaled. Irritating to eyes, respiratory system and skin.

Directions

1. Suspend 55 g of the medium in one liter of purified water.
2. Heat with frequent agitation until the medium reaches the boiling point.
3. AVOID OVERHEATING. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light pink beige.

Prepared Appearance: Prepared medium is bright red to red-orange, trace to slightly hazy.

Expected Cultural Response: Cultural response at 35°C after 20 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	partial to complete inhibition	yellow colonies
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	yellow colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	red colonies w/ black centers
<i>Shigella flexneri</i> ATCC® 12022	growth	red colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Stool specimens or rectal swabs may be plated directly. Selective enrichment broths, such as Selenite Broth or Tetrathionate Broth, may be use prior to streaking.^{5,6} For specific procedures refer to appropriate references.

Results

Degradation of xylose, lactose, and sucrose generates acid products, causing a color change in the colonies and in the medium from red to yellow.

Hydrogen sulfide production under alkaline conditions results in colonies with black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.

Lysine decarboxylation, in the absence of lactose and sucrose fermentation, results in a reversion to an alkaline condition. This alkaline condition causes the color of the medium to change back to red.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Red, false-positive colonies may occur with *Proteus* and *Pseudomonas*.
3. Incubation in excess of 48 hours may lead to false-positive results.

Packaging

XLD Agar	Code No. 7166A	500 g
	7166B	2 kg
	7166C	10 kg

References

1. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars: new media for isolation of enteric pathogens. *Am. J. Clin. Pathol.* **44**(4):471-475.
2. **Rollender, W., O. Beckford, R. D. Belsky, and B. Kostroff.** 1969. Comparison of xylose lysine deoxycholate agar and MacConkey agar for the isolation of *Salmonella* and *Shigella* from clinical specimens. *Tech. Bull. Reg. Med. Tech.* **39**(1):8-10.
3. **Pollock, H. M., and B. J. Dahlgren.** 1974. Clinical evaluation of enteric media in the primary isolation of *Salmonella* and *Shigella*. *Appl Microbiol.* **27**(1):197-201.
4. **Bhat, P., and D. Rajan.** 1975. Comparative evaluation of desoxycholate citrate medium and xylose lysine desoxycholate medium in the isolation of shigellae. *Am. J. Clin. Pathol.* **64**:399-404.
5. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
6. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

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XLT4 AGAR (7517)

Intended Use

XLT4 Agar is used with Tergitol 4 for the isolation of non-typhi *Salmonella* spp.

Product Summary and Explanation

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 of *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 Miller and Tate evaluated the sensitivity of the medium by detecting and isolating *Salmonella* using fecal-contaminated farm samples containing high numbers of competing bacteria. In proceeding studies, Miller^{2,3} and Tate⁴ reported that XLT4 Agar significantly improved the recovery of non-typhi *Salmonella* from chicken and farm environmental drag-swab samples. XLT4 Agar can be used clinically to screen stool samples for non-typhoid *Salmonella*.⁵

Principles of the Procedure

Enzymatic Digest of Animal Tissue is the source of complex nitrogen compounds in XLT4 Agar. Yeast Extract is added as a source of vitamins and cofactors. Differentiation of *Salmonella* from other organisms that also grow on this medium is based on fermentation of Xylose, Lactose and Sucrose, decarboxylase 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. Phenol Red is added as an indicator of pH changes, resulting from fermentation and decarboxylation reactions. Agar is the solidifying agent. Tergitol 4 is added to inhibit growth of non-*Salmonella* organisms.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	4.6 g
Xylose	3.75 g
L-Lysine.....	5 g
Lactose	7.5 g
Sucrose.....	7.5 g
Sodium Chloride	5 g
Yeast Extract.....	3 g
Phenol Red	0.08 g
Ferric Ammonium Citrate.....	0.8 g
Sodium Thiosulfate	6.8 g
Agar	15 g
Final pH: 7.4 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Tergitol 4, 4.6 mL

Precautions

1. For In Vitro Diagnostic Use.
2. HARMFUL. May be harmful if swallowed or inhaled. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 59 g of the medium and 4.6 mL of Tergitol 4 in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. AVOID OVERHEATING. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light pink-tan.

Prepared Appearance: Prepared medium is orange-red and clear to trace hazy.

Expected Cultural Response: Cultural response on XLT4 Agar, with the addition of Tergitol 4, at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	partial inhibition	-----
<i>Escherichia coli</i> ATCC® 25922	partial-complete inhibition	yellow to yellow-red colonies
<i>Proteus mirabilis</i> ATCC® 12453	Inhibited	-----
<i>Staphylococcus aureus</i> ATCC® 25923	Inhibited	-----
<i>Shigella flexneri</i> ATCC® 12022	good growth	red colonies
<i>Salmonella typhimurium</i> ATCC® 14028	good growth	red colonies w/ black centers

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate a suitable *Salmonella* enrichment broth, e.g. Tetrathionate Broth, and incubate at 35°C for 18 - 24 hours. Following enrichment, subculture onto XLT4 Agar. Streak for isolation. Incubate plates aerobically at 35 ± 2°C. Examine for growth after 18 - 24 and 48 hours incubation.

Results

Typical *Salmonella* colonies (H₂S-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 H₂S-negative *Salmonella* strains appear pink-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* spp. are partially inhibited and colonies appear red.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. XLT4 Agar is intended for detecting and isolating *Salmonella* based on selectivity and colonial characteristics. Presumed *Salmonella* colonies must be confirmed biochemically and/or immunologically.

Packaging

XLT4 Agar	Code No.	7517A	500 g
		7517B	2 kg
		7517C	10 kg

References

1. **Miller, R. G., and C. R. Tate.** 1990. XLT4: A highly selective plating medium for the isolation of *Salmonella*. The Maryland Poultryman, April:2-7.
2. **Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Schemer.** 1991. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Science. **70**:2429-2432.
3. **Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Schemer.** 1992. *Erratum*. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Science. **71**:398.
4. **Tate, C. R., R. G. Miller, and E. T. Mallinson.** 1992. Evaluation of two isolation and two non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag-swab samples. J. Food Prot. **55**:964-967.
5. **Dusch, H., and M. Altwegg.** 1995. Evaluation of five new plating media for the isolation of *Salmonella* species. J. Clin. Microbiol. **33**: 802-804.

Technical Information

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YEAST ENRICHED PEPTONE (7208)

Intended Use

Yeast Enriched Peptone is a blend of an enzymatic digest of casein and yeast extract for use in preparing microbiological culture media.

Product Summary and Explanation

Yeast Enriched Peptone is a mixture of casein and yeast extract. The composition of Yeast Enriched Peptone is approximately 1/3 yeast extract and 2/3 casein peptone. The addition of yeast extract to casein peptone provides additional sources of vitamins and carbohydrates. Yeast Enriched Peptone is not recommended for use in media for fermentation studies.

Principles of the Procedure

Yeast Enriched Peptone provides nitrogen, vitamins, carbon, and amino acids in microbiological culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and light beige to tan.

Prepared Appearance: (2% wt/vol): Prepared medium is clear, yellow with no or a light precipitate.

pH (2% Solution at 25°C): 6.5 - 7.5

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	fair to good growth

Test Procedure

Refer to appropriate references for specific procedures using Yeast Enriched Peptone. For a complete discussion on the isolation and identification of microorganisms, refer to procedures outlined in the references.^{1,2}

Results

Refer to appropriate references for test results.

Storage

Store sealed bottle containing Yeast Enriched Peptone at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Yeast Enriched Peptone should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to Yeast Enriched Peptone in its intact container when stored as directed.

Packaging

Yeast Enriched Peptone	Code No.	7208A	500 g
		7208B	2 kg
		7208C	10 kg

References

1. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. Vol. 1. American Society for Microbiology, Washington, D.C.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

YEAST EXTRACT (7184)

Intended Use

Yeast Extract is an autolysate of yeast cells used in preparing microbiological culture media.

Product Summary and Explanation

Yeast Extract is the water-soluble portion of autolyzed yeast. The autolysis is carefully controlled to preserve naturally occurring B-complex vitamins. Yeast Extract is prepared and standardized for bacteriological use, and an excellent stimulator of bacterial growth. Yeast Extract is generally employed in the concentration of 0.3% - 0.5%. Yeast Extract is typically prepared by growing baker's yeast, *Saccharomyces* spp., in a carbohydrate-rich plant medium. The yeast is harvested, washed, and resuspended in water, where it undergoes autolysis, i.e., self-digestion using the yeast's enzymes. 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 filtered clear and dried into a powder by spray drying.

Yeast Extract has been successful in culture media for bacterial studies in milk and other dairy products. Several media containing Yeast Extract are specified in standard methods for multiple applications.¹⁻⁴

Principles of the Procedure

Yeast Extract provides vitamins, nitrogen, amino acids, and carbon in microbiological culture media.

Precaution

1. For Laboratory Use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing and light beige to tan.

Prepared Appearance (2% wt/vol): Prepared medium is clear, amber with no or a light precipitate.

pH (2% Solution at 25°C): 6.8 - 7.2

Expected Cultural Response: Cultural response on 2% Peptone Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response
<i>Escherichia coli</i> ATCC 25922	good to excellent growth
<i>Staphylococcus aureus</i> ATCC 25923	poor to fair growth

Test Procedure

Refer to appropriate references for specific procedures using Yeast Extract.

Results

Refer to appropriate references for test results.

Storage

Store sealed container of Yeast Extract at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. Yeast Extract should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to Yeast Extract in its intact container when stored as directed.

Packaging**Yeast Extract**

Code No.	7184A	500 g
	7184B	2 kg
	7184C	10 kg

References

1. **U.S. Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R.T. (ed.).** Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D. C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

YERSINIA SELECTIVE AGAR (7257)

Intended Use

Yersinia Selective Agar is used with cefsulodin and novobiocin for the isolation of *Yersinia enterocolitica*.

Product Summary and Explanation

Yersinia enterocolitica is a significant food or water borne enteric pathogen.^{1,2} Yersinia Selective Agar is a selective and differential medium supporting good growth of *Y. enterocolitica* and some *Yersinia* spp. The formula is based on Cefsulodin-Irgasan-Novobiocin (CIN) Agar developed by Schiemann.³⁻⁶ In comparison with MacConkey Agar and SS Agar, Schiemann found CIN Agar provided increased inhibition of normal enteric organisms and provided improved direct recovery of *Y. enterocolitica* from feces.⁴ Schiemann later modified his formula to further improve growth and recovery of *Y. enterocolitica*.⁶

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue provide the nitrogen and amino acids in Yersinia Selective Agar. Yeast Extract is the vitamin source in this formula. Selectivity of Yersinia Selective Agar is due to the presence of Sodium Deoxycholate, Sodium Cholate, Crystal Violet, and Irgasan® that markedly inhibit growth of gram-positive and many gram-negative organisms. Supplementation with Cefsulodin and Novobiocin improves inhibition of normal enteric organisms. Differentiation is based on Mannitol fermentation. Organisms capable of fermenting Mannitol lower the pH around the colony, allowing absorption of Neutral Red and turning the colony a red color. Due to the localized pH decrease, a zone of precipitated bile may also be present. Organisms that do not metabolize Mannitol to acid end products will form colorless, translucent colonies. Sodium Chloride maintains the osmotic balance of the medium. Sodium Pyruvate and Magnesium Sulfate stimulate organism growth. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Yeast Extract.....	2 g
Mannitol	20 g
Sodium Deoxycholate	0.5 g
Sodium Cholate	0.5 g
Sodium Chloride	1 g
Sodium Pyruvate.....	2 g
Magnesium Sulfate	0.01 g
Neutral Red	0.03 g
Crystal Violet.....	0.001 g
Irgasan®	0.004 g
Agar	13.5 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobial / 10 mL

Cefsulodin	4 mg
Novobiocin	2.5 mg

Precaution

1. For In Vitro Diagnostic Use.

Directions

1. Suspend 59.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool sterilized medium to 45 - 50°C and aseptically add 10 mL of a sterilized aqueous solution containing 4 mg of Cefsulodin and 2.5 mg of Novobiocin.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light pink-beige to beige.

Prepared Appearance: Prepared medium is red-orange and clear to trace hazy.

Expected Cultural Response: Cultural response on Yersinia Selective Agar at 35°C after 24 - 48 hours incubation.

Microorganism	Response
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited
<i>Escherichia coli</i> ATCC® 25922	inhibited
<i>Proteus mirabilis</i> ATCC® 12453	inhibited
<i>Yersinia enterocolitica</i> ATCC® 9610	growth
<i>Yersinia enterocolitica</i> ATCC® 27729	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on the isolation and identification of *Yersinia*, refer to specific procedures.

Results

Yersinia enterocolitica colonies appear translucent or translucent with dark pink centers. Colony edges are entire or irregular. After 48 hour incubation, colonies appear dark pink with a translucent border and may be surrounded by a zone of precipitated bile. Growth of non-*Yersinia* organisms is markedly to completely inhibited.

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *Yersinia* spp.
2. Some strains of normal enteric organisms may be encountered that are not inhibited or only partially inhibited on complete medium, such as *Citrobacter freundii*, *Serratia liquefaciens*, and *Enterobacter agglomerans*.
3. Growth of *Yersinia frederiksenii*, *Y. kristensenii*, *Y. pseudotuberculosis* and *Y. intermedia* is not inhibited on complete medium. Colonies of these organisms must be differentiated from *Y. enterocolitica* on the basis of additional characteristics.

Packaging

Yersinia Selective Agar	Code No.	7257A	500 g
		7257B	2 kg
		7257C	10 kg

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Vanderzant, C. and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food. 3rd ed. American Public Health Association, Washington, D.C.
3. **Schiemann, D. A.** 1979. Synthesis of a selective agar medium for *Yersinia enterocolitica*. Can. J. Microbiol. **35**:1298-1304.
4. **Schiemann, D. A.** 1980. *Yersinia enterocolitica*: Observations on some growth characteristics and response to selective agents. Can. J. Microbiol. **26**:1232-1240.
5. **Devenish, J. A., and D. A. Schiemann.** 1981. An abbreviated scheme for identification of *Yersinia enterocolitica* isolated from food enrichments on CIN (cefsulodin-irgasan-novobiocin) agar. Can. J. Microbiol. **27**:937-941.
6. **Schiemann, D. A.** 1982. Development of a two-step enrichment procedure for recovery of *Yersinia enterocolitica* from food. Appl. Environ. Microbiol. **43**:14-27.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

YM AGAR (7525)

Intended Use

YM Agar is used for the cultivation of yeasts, molds, and other aciduric organisms.

Product Summary and Explanation

YM Agar is prepared according to the formulation published by Wickerham.¹⁻³ YM Agar was formulated for the selective isolation of yeasts from mixed cultures containing bacteria and molds. Media selectivity may be enhanced through acidification or through addition of selective agents. YM Agar should be sterilized without pH adjustment and sterile acid added to the medium cooled to 45 - 50°C. Acidified YM Agar should not be heated. Antibiotics may be aseptically added to sterile media. Other fungistatic materials may be added to YM Agar to eliminate molds and permit enumeration of yeasts to mixed populations.

Principles of the Procedure

Enzymatic Digest of Gelatin is a nitrogen and amino acid source in YM Agar. Yeast Extract provides trace elements and vitamins. Malt Extract is a source of carbon, protein, and nutrients. Dextrose is an energy source. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	5 g
Yeast Extract.....	3 g
Malt Extract.....	3 g
Dextrose.....	10 g
Agar	20 g

Final pH: 6.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 41 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Optional

If desired, acidify YM Agar to pH 3.0 – 4.0 by adding sterile 10% HCl, Tartaric Acid, or 10% Citric Acid. Selective agents, e.g., penicillin (20 units per mL final concentration) or streptomycin (40 micrograms per mL final concentration) may be added to the medium after sterilization using aseptic technique.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and pale to light yellow.

Expected Cultural Response: Cultural response on YM Agar at 25 - 30°C after 2 - 7 days incubation.

Microorganism	Response	Reactions
<i>Aspergillus niger</i> ATCC® 16404	growth	white cottony to black powdery
<i>Candida albicans</i> ATCC® 10231	growth	off-white to beige pasty
<i>Lactobacillus fermentum</i> ATCC® 9338	growth	---
<i>Microsporium canis</i> ATCC® 36299	growth	white cottony-powdery
<i>Penicillium roquefortii</i> ATCC® 10110	growth	grey powdery
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth	off-white to beige, pasty

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate YM Agar with the appropriate sample for the presence of yeasts, molds, or aciduric microorganisms.
2. Incubate at $30 \pm 2^{\circ}\text{C}$ for 2 to 7 days.

Results

Examine plates for growth. Record YM Agar results as colony forming units (CFU) per volume of sample.

Storage

Store sealed bottle containing the dehydrated medium at $2 - 30^{\circ}\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Acidified YM Agar should not be reheated.

Packaging

YM Agar	Code No.	7525A	500 g
		7525B	2 kg
		7525C	10 kg

References

1. **1951.** U. S. Dept. Agricult. Tech. Bull. No. 1029.
2. **1939.** J. Tropical Med. Hyg. **42**:176.
3. **Jong, S. C., and M. J. Edwards.** 1991. American Type Culture Collection Catalog of filamentous fungi. 18th ed. American Type Collection, Rockville, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.

YM BROTH (7363)

Intended Use

YM Broth is used for the cultivation of fungi.

Product Summary and Explanation

YM Broth is prepared according to the formulation 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. To favor isolation of fermentative species, add a layer of sterile paraffin oil 1 cm deep on the surface of the inoculated broth. Cultures should be incubated until growth appears, and then streaked 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 to 2 days. This favors yeast recovery while preventing sporulation of molds.

Media selectivity may be enhanced through acidification or addition of selective agents. YM Broth may be acidified prior to sterilization.

Principles of the Procedure

Enzymatic Digest of Gelatin is a nitrogen and amino acid source in YM Broth. Yeast Extract provides trace elements and vitamins. Malt Extract is a source of carbon, protein, and nutrients. Dextrose is an energy source.

Formula / Liter

Enzymatic Digest of Gelatin	5 g
Malt Extract	3 g
Dextrose.....	10 g
Yeast Extract.....	3 g

Final pH: 6.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful if swallowed. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 21 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Optional

If desired, acidify YM Broth to pH 3.0 - 4.0 by adding sterile 10% HCl, Tartaric Acid, or 10% Citric Acid. Selective agents, e.g., penicillin (20 units per mL final concentration) or streptomycin (40 micrograms per mL final concentration) may be added to the medium after sterilization using aseptic technique.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and beige.

Prepared Appearance: Prepared medium is clear to trace hazy and light yellow to gold.

Expected Cultural Response: Cultural response, after inoculation in YM Broth at 25 - 30°C for 2 - 7 days incubation.

Microorganism	Response
<i>Aspergillus niger</i> ATCC® 16404	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Microsporum canis</i> ATCC® 36299	growth
<i>Penicillium roquefortii</i> ATCC® 10110	growth
<i>Saccharomyces cerevisiae</i> ATCC® 9763	growth
<i>Trichophyton mentagrophtes</i> ATCC® 9533	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate YM Broth with appropriate samples to evaluate the presence of yeasts, molds, or aciduric microorganisms.
2. Incubate at 30 ± 2°C for 18 - 72 hours.

Results

Examine tubes for growth. Record YM Broth results as growth or no growth.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

YM Broth	Code No.	7363A	500 g
		7363B	2 kg
		7363C	10 kg

References

1. 1951. U. S. Dept. Agricult. Tech. Bull. No. 1029.
2. 1939. J. Tropical Med. Hyg. 42:176.
3. Jong, S. C., and M. J. Edwards. 1991. American Type Culture Collection Catalog of filamentous fungi. 18th ed. American Type Collection, Rockville, MD.

Technical Information

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Acumedia Dehydrated Culture Media

Listed in alphabetical order

A-1 Medium	Dermatophyte Test Medium
Agar, Bacteriological	Dextrose Tryptone Agar
APT Agar	Dextrose Tryptone Broth
Azide Dextrose Broth	Dichloran Glycerol (DG-18) Agar Base
Bacillus Cereus Agar Base	Dipeptone
Baird Parker Agar	DNase Test Agar
BCYE Agar (Legionella Agar)	DNase Test Agar w/ Methyl Green
Beef Extract Powder	DRBC Agar
Beta-SSA Agar	EC Medium
BIGGY Agar	EC Medium w/ MUG
Bile Esculin Agar	EC Medium, Modified
Bile Esculin Azide Agar	Elliker Broth
Bile Salts Mixture # 3	EMB Agar (Holt, Harris & Teague)
Bismuth Sulfite Agar	EMB Agar, Levine
Blood Agar Base No.2	Eugonic Agar
Blood Agar Base w/ Low pH	Fastidious Anaerobe Agar
Blood Agar Base, Improved	Fluid Thioglycollate Medium
Blood Agar Base, pH 7.4	Fraser Broth
Brain-Heart Infusion Agar	Fraser Broth Base
Brain-Heart Infusion Broth	Fungisel Agar
Brain-Heart Infusion Solids	GC Agar
Brain-Heart Infusion w/o Dextrose	Gelatin
Brilliant Green Agar	GN Broth (Hajna)
Brilliant Green Agar w/ Sulfadiazine	HC Agar Base
Brilliant Green Agar w/ Sulfapyridine	Heart Infusion Agar
Brilliant Green Bile Agar	Heart Infusion Broth
Brilliant Green Bile Broth 2%	Hektoen Enteric Agar
Brilliant Green Bile Broth 2% w/ MUG	Hemoglobin Powder
Brucella Agar	Inhibitory Mold Agar
Brucella Broth	Kligler Iron Agar
Buffered Listeria Enrichment Broth	Lactobacilli MRS Agar
Buffered Peptone Water	Lactobacilli MRS Broth
Campy Blood Free Selective Medium (CCDA)	Lactobacillus Selective Agar Base
Campy Selective Agar Base (Preston)	Lactose Broth
Campylobacter Enrichment Broth	Lauryl Sulfate Broth
Cary and Blair Transport Medium	Lauryl Sulfate Broth w/ MUG
Cary and Blair Trans Med, Mod w/ Phenol Red	LB Agar (Lennox L Agar)
Casein, Acid Hydrolysate	LB Broth (Lennox L Broth)
Casman Medium Base	Lethen Agar Base
CDC Anaerobe Agar	Lethen Agar Base, Modified
Cetrimide Agar	Lethen Broth Base
Charcoal Agar	Lethen Broth Base, Modified
CHO Medium Base	Listeria Enrichment Broth
CLED Agar	Littman Agar
Clostridium Difficile Agar	Lowenstein-Jensen Medium
Columbia Blood Agar Base	LPM Agar
Columbia Broth	Luria Agar (Miller's LB Agar)
Columbia CNA Agar	Luria Broth Base (Miller's LB Broth)
Cooked Meat Medium	Lysine Iron Agar
D/E Neutralizing Agar	M-Broth
D/E Neutralizing Broth	m-Enterococcus Agar
Deoxycholate Agar	m-FC Agar
Deoxycholate Citrate Agar	m-FC Broth

m-Green Yeast and Fungi Broth	Sabouraud BHI Agar
m-TEC Agar	Sabouraud Dextrose Agar
m-TGE Broth	Sabouraud Dextrose Agar w/ Chloramphenicol
M17 Broth Base	Sabouraud Dextrose Agar w/ Lec & Tween 80
MacConkey Agar	Sabouraud Dextrose Agar, Emmons
MacConkey Agar w/o Crystal Violet	Salmonella Shigella Agar
MacConkey Agar w/o Crystal Violet & Salt	Schaedler Agar
MacConkey Agar w/ Sorbitol	Schaedler Broth
MacConkey Agar, CS	Selective Strep Agar, Modified #2
MacConkey Agar, Modified	Selenite Broth
MacConkey Broth	Selenite Cystine Broth
Malonate Broth	SIM Medium
Malt Agar	Simmons Citrate Agar
Malt Extract	Skim Milk
Malt Extract Agar	Soy Peptone Yeast Extract Agar
Mannitol Salt Agar	Standard Methods Agar
MCP Agar	Staph Selective Agar
Middlebrook 7H10 Agar	Staphylococcus Agar # 110
Middlebrook 7H11 Agar	Sterility Test Broth
MIO Medium	TAT Broth
Mitis Salivarius Agar	TCBS Agar
Motility Test Agar	Tergitol 7 Agar
MRSA Agar Base	Tetrathionate Broth Base
MR-VP Broth	Thioglycollate Medium w/o Indicator
Mueller Hinton Agar	Todd Hewitt Broth
Mycobiotic Agar	Tomato Juice Agar
Mycological Agar	Triple Sugar Iron Agar
Nutrient Agar	Tryptic Soy Agar
Nutrient Agar 1.5%	Tryptic Soy Agar w/ Lec & Tween 80
Nutrient Broth	Tryptic Soy Broth
Nutrient Gelatin	Tryptone
Orange Serum Agar	Tryptone Glucose Extract Agar
Ox bile (Ox gall)	Tryptose Agar
Oxford Listeria Agar Base	Tryptose Blood Agar Base
Pancreatic Digest of Casein (Peptone C)	Tryptose Broth
Pancreatic Digest of Gelatin (Peptone G)	Tryptose Phosphate Broth
Papaic Digest of Soybean Meal (Peptone S)	Universal Beer Agar
Peptic Digest of Animal Tissue (Peptone A)	Universal Pre-Enrichment Broth
Peptone Water	Urea Agar Base
Phenol Red Broth Base	UVM Mod Listeria Enrichment Broth
Phenylethanol Agar	Violet Red Bile Agar
Phosphate Buffer, pH 7.2	Violet Red Bile Agar w/ MUG
Potato Dextrose Agar	Violet Red Bile Glucose Agar
Potato Dextrose Agar with Lec & Tween 80	Vogel and Johnson Agar
Potato Dextrose Broth	Wilkins-Chalgren Agar
Potato Infusion Agar	Wilkins-Chalgren Broth
Presence-Absence Broth	W-L Nutrient Medium
Pseudomonas F Agar	XL Agar Base
Pseudomonas Isolation Agar	XLD Agar
Pseudomonas Isolation Broth	XLT4 Agar
Pseudomonas P Agar	Yeast Enriched Peptone
Purple Lactose Agar	Yeast Extract
R2A Agar	Yersinia Selective Agar
Rappaport-Vassiliadis (MSRV) Medium	YM Agar
Semisolid Modified	YM Broth
Rappaport-Vassiliadis R10 Broth	