Bacto® DCLS Agar

Intended Use

Bacto DCLS Agar is used for isolating gram-negative enteric bacilli.

Also Known As

DCLS is an abbreviation for Desoxycholate Citrate Lactose Saccharose Agar.

Summary and Explanation

DCLS Agar is a modification of SS Agar and the Desoxycholate Citrate Agar described by Leifson[1]. Coliform organisms capable of fermenting lactose or sucrose are generally inhibited. Gram positive bacteria are suppressed.

While studying enteric pathogens on Endo medium, Holt-Harris and Teague[2] used lactose and sucrose in the development of a nutrient agar containing methylene blue and eosin. Some coliforms ferment sucrose more readily than lactose. The addition of sucrose (saccharose) allows nonpathogenic sucrose-fermenting organisms to produce red colonies. The red colonies are easily recognized, reducing the number of false positive reactions.

Principles of the Procedure

Beef Extract and Proteose Peptone No. 3 provide nitrogen, vitamins and amino acids. Lactose and Saccharose (sucrose) provide fermentable carbohydrates. Sodium Citrate, Sodium Thiosulfate and Sodium Desoxycholate are selective agents. Bacto Agar is the solidifying agent. Neutral Red is the indicator.

Formula

DCLS Agar

Formula Per Liter

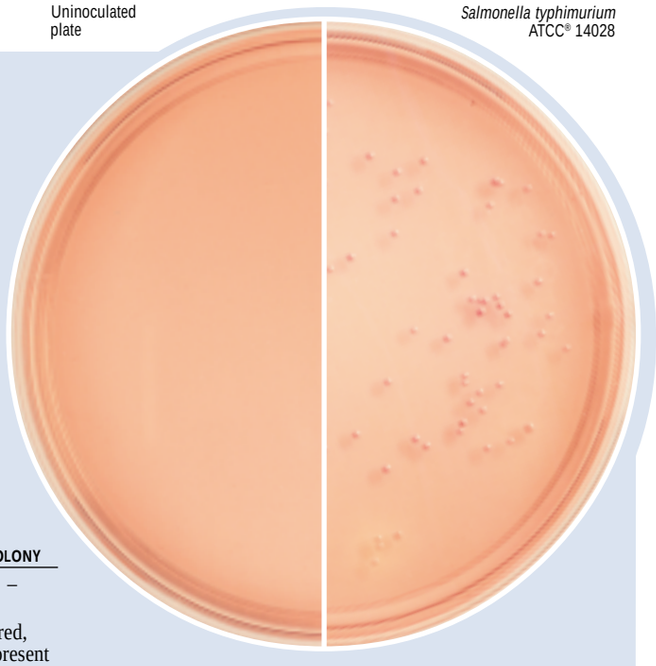
Bacto Beef Extract	3 g
Bacto Proteose Peptone No.3	7 g
Bacto Lactose	5 g
Bacto Saccharose (sucrose)	5 g
Sodium Citrate	10 g
Sodium Thiosulfate	5 g
Sodium Desoxycholate	2.5 g
Bacto Agar	12 g
Neutral Red	0.03 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to light pink, free-flowing, homogeneous.
Solution:	4.95% solution, soluble in distilled or deionized water upon boiling. After boiling, orange-red, clear to very slightly opalescent, without significant precipitation.
Prepared Medium:	Orange-red, slightly opalescent.
Reaction of 4.95% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	COLOR OF GROWTH	COLONY
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition	–
Escherichia coli	25922*	100-1,000	marked to complete inhibition	red, if present
Salmonella typhimurium	14028*	100-1,000	good	colorless to pink
Shigella flexneri	12022*	100-1,000	fair to good	colorless to pink

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

DCLS Agar

Materials Required But Not Provided

Glassware
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 49.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. DO NOT AUTOCLAVE.
3. Cool to 50-55°C.
4. Dispense into sterile Petri dishes, or as desired.
5. Allow prepared medium to dry for about 2 hours with the covers partially removed.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of enteric pathogens from clinical specimens, refer to the procedures described in appropriate references.[3,4]

Results

Typical coliforms that rapidly ferment sucrose and/or lactose will form red, opaque colonies. *Shigella* and *Salmonella* species will produce colorless to slightly pink, transparent colonies.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. DO NOT AUTOCLAVE MEDIUM. DO NOT OVERHEAT.
3. DCLS Agar is intended for selective use and should be inoculated in parallel with nonselective media.
4. Colonies suspected of being enteric pathogens must be confirmed biochemically and, if required, serologically.

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. Bacteriol. **40**:581-599.
2. **Holt-Harris, J. E., and O. Teague.** 1916. A new culture medium for the isolation of Bacillus typhosus from stools. J. Infect. Dis. **18**:596-601.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.

Packaging

DCLS Agar	500 g	0759-17

Bacto® D/E Neutralizing Agar
Bacto D/E Neutralizing Broth

Intended Use

Bacto D/E Neutralizing Agar is used for neutralizing and determining the bactericidal activity of antiseptics and disinfectants.

Bacto D/E Neutralizing Broth is used for determining the bactericidal activity of antiseptics and disinfectants based on neutralizing the chemical and detecting organisms remaining after treatment.

Also Known as

D/E Neutralizing Agar and Broth are also known as Dey-Engley Neutralizing Agar and Broth.

Summary and Explanation

D/E Neutralizing media, developed by Dey and Engley,[1] neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals. D/E Neutralizing media neutralize higher concentrations of residual antimicrobials when compared with other standard neutralizing formulations such as Letheen media, Thioglycollate media, and Neutralizing Buffer.[2,3]

Complete neutralization of disinfectants is important because disinfectant carryover can cause a false no-growth test result. D/E Neutralizing media effectively neutralize the inhibitory effects 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 media are recommended for use in disinfectant evaluation, environmental sampling (swab and contact plate methods) and the testing of water-miscible cosmetics in accordance with Cosmetic, Toiletry and Fragrance Association (CTFA) guidelines.[6]

Principles of the Procedure

D/E Neutralizing Agar and Broth contain Tryptone which provides the carbon and nitrogen sources required for growth of a wide variety of

organisms. Yeast Extract provides vitamins and cofactors required for growth and additional nitrogen and carbon. Dextrose is a source of fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and gluteraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol.[11] Brom Cresol Purple is used as a colorimetric indicator to demonstrate the production of acid from the fermentation of dextrose.

D/E Neutralizing Agar uses Bacto Agar as a solidifying agent.

Formula

D/E Neutralizing Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	2.5 g
Bacto Dextrose	10 g
Sodium Thioglycollate	1 g
Sodium Thiosulfate	6 g
Sodium Bisulfite	2.5 g
Polysorbate 80	5 g
Lecithin (Soy Bean)	7 g
Bacto Agar	15 g
Bacto Brom Cresol Purple	0.02 g

Final pH 7.6 ± 0.2 at 25° C

D/E Neutralizing Broth

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	2.5 g
Bacto Dextrose	10 g
Sodium Thioglycollate	1 g
Sodium Thiosulfate	6 g
Sodium Bisulfite	2.5 g
Polysorbate 80	5 g
Lecithin (Soy Bean)	7 g
Bacto Brom Cresol Purple	0.02 g

Final pH 7.6 ± 0.2 at 25° C

Precautions

1. For Laboratory Use.
2. **D/E Neutralizing Agar**
   D/E Neutralizing Broth

   HARMFUL. MAY CAUSE SENSITIZATION BY INHALATION. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh

User Quality Control

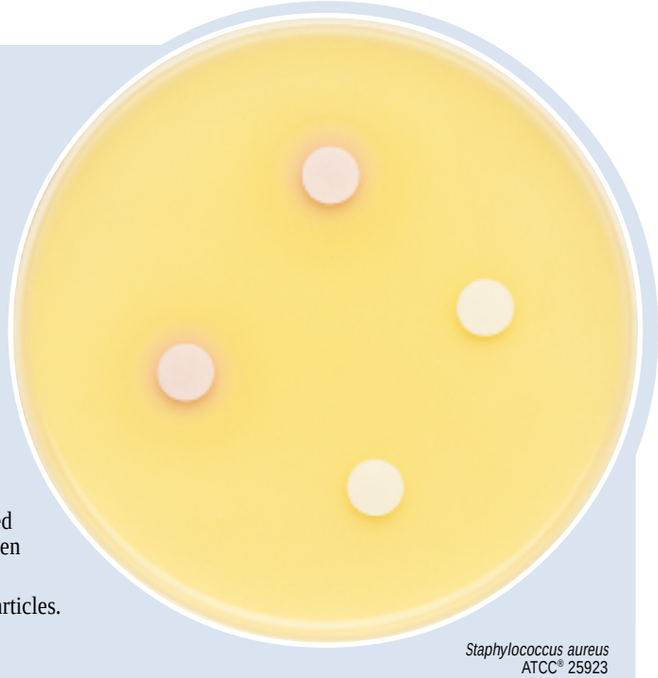
Identity Specifications

D/E Neutralizing Agar

Dehydrated Medium:	Bluish-grey, homogeneous, appears moist and lumpy.
Solution:	5.4% solution, soluble in distilled or deionized water on boiling. Lavender, opaque with an even suspension of fine particles.
Prepared Medium:	Lavender, opaque with a fine precipitate.
Reaction of 5.4% Solution at 25°C:	7.6 ± 0.2

D/E Neutralizing Broth

Dehydrated Medium:	Bluish-grey, homogeneous, appears moist and lumpy.
Solution:	3.9% solution, soluble in distilled or deionized water on warming. Purple, opaque with an even suspension of fine particles.
Prepared Medium:	Purple, opaque with an even suspension of particles.
Reaction 3.9% Solution at 25°C:	7.6 ± 0.2



Staphylococcus aureus
ATCC® 25923

Cultural Response

D/E Neutralizing Agar: Neutralization Test

Prepare medium per label directions. Inoculate 50 ml of D/E Neutralizing Agar with 0.1 ml of a heavy suspension of each test organism and dispense into 150 x 15 mm Petri dishes of D/E Neutralizing Agar and Plate Count Agar. Place 1/2 inch sterile blank disks on each plate. Dispense 0.1 ml of each disinfectant solution onto two disks per medium. Incubate at 35 ± 2°C for 40-48 hours. D/E Neutralizing Agar should exhibit no zones of inhibition or zones significantly smaller than those found on Plate Count Agar.

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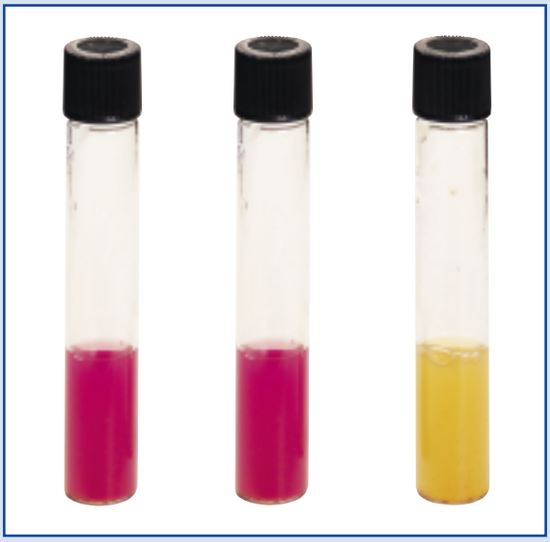
User Quality Control cont.

Cultural Response

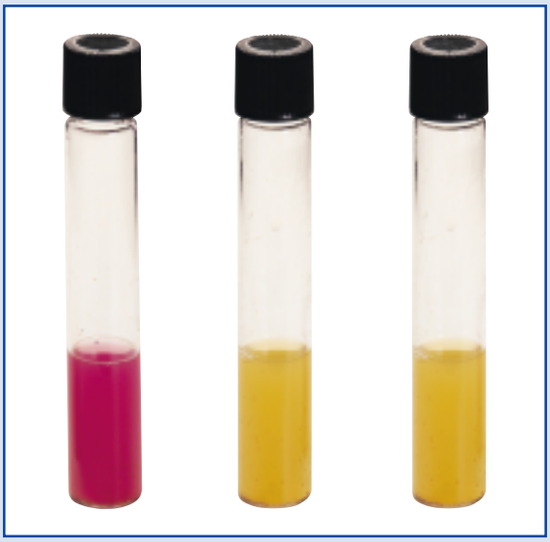
D/E Neutralizing Broth: Toxicity Test

Prepare medium per label directions with and without added disinfectants. Inoculate with 100-1,000 CFU of test organism. Incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis	6633	100-1,000	good
Escherichia coli	25922	100-1,000	good
Pseudomonas aeruginosa	27853	100-1,000	good
Salmonella typhimurium	14028	100-1,000	good
Staphylococcus aureus	25923	100-1,000	good



Uninoculated tube | *Bacillus subtilis* ATCC® 6633 | *Escherichia coli* ATCC® 25922



Pseudomonas aeruginosa ATCC® 27853 | *Salmonella typhimurium* ATCC® 14028 | *Staphylococcus aureus* ATCC® 25923

air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

D/E Neutralizing Agar
D/E Neutralizing Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave

Method of Preparation

D/E Neutralizing Agar

1. Suspend 54 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve.
3. Autoclave at 121°C for 15 minutes.

D/E Neutralizing Broth

1. Suspend 39 grams in 1 liter of distilled of deionized water.
2. Heat to dissolve.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

D/E Neutralizing Agar and D/E Neutralizing Broth are used in a variety of procedures. Consult appropriate references for further information.[6]

Results

Refer to appropriate references and procedures for results.

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. (ed.).** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

Packaging

D/E Neutralizing Agar	500 g	0686-17
	10 kg	0686-08
D/E Neutralizing Broth	500 g	0819-17

Bacto® DNase Test Agar
Bacto DNase Test Agar w/Methyl Green

Intended Use

Bacto DNase Test Agar and Bacto DNase Test Agar w/Methyl Green are used for differentiating microorganisms based on deoxyribonuclease activity.

Summary and Explanation

In 1956, Weckman and Catlin[1] showed a correlation between increased DNase activity of *Staphylococcus aureus* and positive coagulase activity. They suggested that DNase activity could be used to identify potentially pathogenic staphylococci. DiSalvo[2] confirmed their results by obtaining excellent correlation between the coagulase and DNase activity of staphylococci isolated from clinical specimens. Jeffries, Holtman and Guse[3] incorporated DNA in an agar medium to study DNase production by bacteria and fungi. Polymerized DNA precipitates in the presence of 1N HCl, making the medium opaque. Organisms that degrade DNA produce a clear zone around an inoculum streak. Fusillo and Weiss[4] studied the calcium requirements of staphylococci for DNase production and concluded that additional calcium was unnecessary when a complete nutritive medium was used.

Kurnick[5] showed that methyl green combines with highly polymerized DNA at pH 7.5. When combination does not take place, the color fades,

User Quality Control

Identity Specifications

DNase Test Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 7.3 ± 0.2

DNase Test Agar w/Methyl Green

Dehydrated Appearance:	Light beige with a slight green tint, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in distilled or deionized water on boiling. Solution is green, very slightly to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Green, slightly opalescent, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 7.3 ± 0.2

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Staphylococcus aureus
ATCC® 25923
DNase Test Agar

creating a clear zone around the growth. Applying this principle, Smith, Hancock and Rhoden[6] modified DNase Test Agar with added methyl green to detect staphylococci, streptococci and *Serratia*. When using DNase Test Agar w/Methyl Green, acid does not have to be added to the plate.

Mannitol fermentation can be determined simultaneously with DNase production by adding 10 grams of mannitol and 0.025 grams of phenol red to the DNase Test Agar prior to sterilization.[7]

Principles of the Procedure

Tryptose is a source of nitrogen, amino acids and carbon. Deoxyribonucleic Acid enables the detection of DNase that depolymerizes DNA. Sodium Chloride provides essential ions while maintaining osmotic balance. Methyl Green is a colorimetric indicator. Bacto Agar is a solidifying agent.

Formula

DNase Test Agar

Formula Per Liter

Bacto Tryptose	20 g
Deoxyribonucleic Acid	2 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

DNase Test Agar w/Methyl Green

Formula Per Liter

Bacto Tryptose	20 g
Deoxyribonucleic Acid	2 g
Sodium Chloride	5 g
Bacto Agar	15 g
Methyl Green	0.05 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

DNase Test Agar
DNase Test Agar w/Methyl Green

Materials Required but not Provided

Glassware
Autoclave
1N Hydrochloric acid (DNase Test Agar)

Method of Preparation

1. Suspend 42 grams of either DNase Test Agar or DNase Test Agar w/Methyl Green in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

User Control Quality cont.

Cultural Response

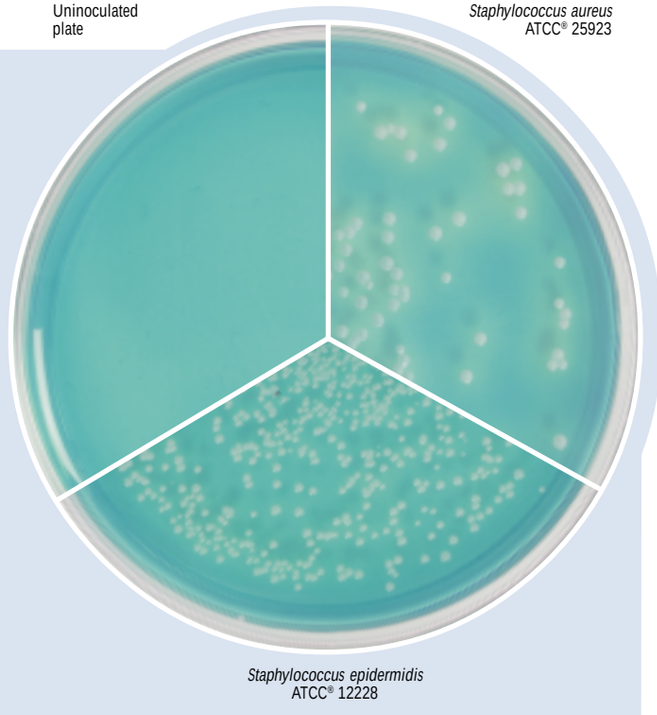
DNase Test Agar
DNase Test Agar w/Methyl Green

Prepare DNase Test Agar or DNase Test Agar w/Methyl Green per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 and up to 48 hours. Flood DNase Test Agar (only) with 1N hydrochloric acid prior to observing DNase activity.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	DNASE TEST
Serratia marcescens	8100*	100-1,000	good	+
Staphylococcus aureus	25923*	100-1,000	good	+
Staphylococcus epidermidis	12228*	100-1,000	good	–
Streptococcus pyogenes	19615*	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.

*****These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate plates by spotting or streaking with a heavy inoculum of the 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. DNase Test Agar: Flood the plates with 1N hydrochloric acid.

   DNase Test Agar w/Methyl Green: Do NOT flood with 1N hydrochloric acid.
4. Observe for clearing around the spot or streak. Record results.

Results

DNase Test Agar:	A zone of clearing around the spot or streak indicates DNase activity.
DNase Test Agar w/Methyl Green:	A decolorized zone (halo) around the spot or streak indicates DNase activity.

Limitations of the Procedure

1. The composition of the culture medium, the degree of aeration, pH, temperature and incubation period are important factors influencing DNase activity in the culturing and testing the micrococci.[7]

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 for 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.

Packaging

DNase Test Agar	100 g	0632-15
	500 g	0632-17
DNase Test Agar w/Methyl Green	100 g	0220-15
	500 g	0220-17

Bacto® DRBC Agar

User Quality Control

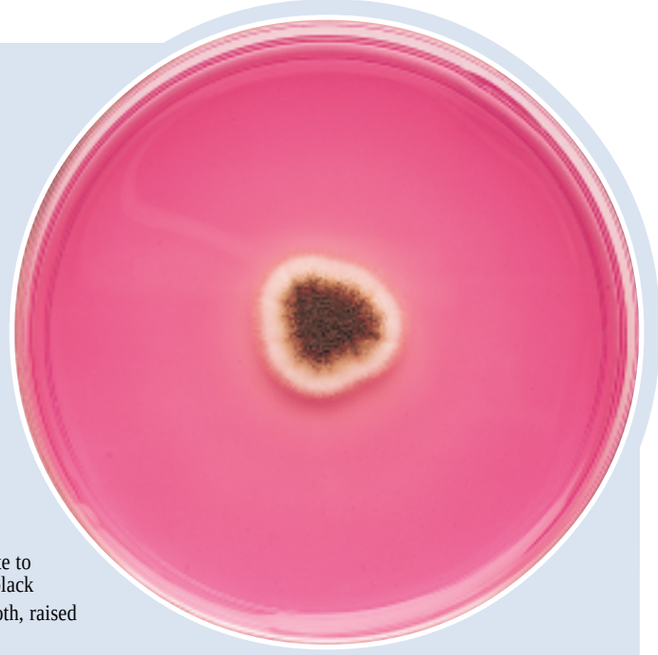
Identity Specifications

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	3.16% solution, soluble in distilled or deionized water upon boiling. Solution is reddish pink, very slightly to slightly opalescent.
Prepared Medium:	Bright pink, very slightly to slightly opalescent.
Reaction of 3.16% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Prepare DRBC Agar per label directions. Inoculate and incubate plates at 25°C for 5 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	1015	Stab	good-colonies white to salt and pepper to black
Candida albicans	10231	100-1,000	good-colonies pink,smooth, raised
Escherichia coli	25922*	1,000-2,000	none to poor
Micrococcus luteus	10240	1,000-2,000	none to poor



Aspergillus niger
ATCC® 1015

The cultures listed are the minimum that should be used for performance testing.
*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use

Bacto DRBC Agar is used for the enumeration of yeasts and molds.

Also Known As

Dichloran Rose Bengal Chloramphenicol Agar

Summary and Explanation

DRBC Agar is based on the 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 that supports good growth of yeasts and molds.

Principles of the Procedure

Proteose Peptone No. 3 provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate is a buffering agent. Magnesium Sulfate is a source of divalent cations and sulfate. The antifungal agent, Dichloran, is added to the medium to reduce colony diameters of spreading fungi. The pH of the medium is reduced from 7.2 to 5.6 for improved inhibition of the spreading fungi.[1] The presence of Rose Bengal in the medium suppresses the growth of bacteria and restricts the size and height of colonies of the more rapidly growing molds. The concentration of Rose Bengal is reduced from 50 µg/ml to 25 µg/ml as found in Rose Bengal Chloramphenicol Agar for optimal performance with Dichloran. Chloramphenicol is included in this medium to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more rapidly growing molds aids in the isolation of slow-growing fungi by preventing their overgrowth by more rapidly growing species. In addition, Rose Bengal is taken up by yeast and mold colonies, which allows these colonies to be easily recognized and enumerated. Reduced recovery of yeasts may be encountered due to increased activity of Rose Bengal at pH 5.6.[1] Bacto Agar is a solidifying agent.

Formula

DRBC Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Dextrose	10 g
Potassium Phosphate Monobasic	1 g
Magnesium Sulfate	0.5 g
Dichloran	0.002 g
Rose Bengal	0.025 g
Chloramphenicol	0.1 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **TOXIC.** MAY CAUSE CANCER. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Nerves, Lymph Glands, Eyes.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

1. Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.
2. Protect medium from light.
3. Store prepared medium in the dark at 2-8°C.

Expiration Date

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

Procedure[2,3]

Materials Provided

DRBC Agar

Materials Required But Not Provided

Peptone Water
Flasks with closures
Distilled or deionized water
Autoclave
Incubator (25°C)

Method of Preparation

1. Suspend 31.6 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Prepare sample for surface inoculation following recommended guidelines.[2,3] The use of 0.1% Peptone Water as the diluent is recommended.

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 the plate using a sterile, bent-glass rod.
2. Incubate plates upright at 22-25°C. Examine for growth of yeasts and molds after 3, 4 and 5 days incubation.

Results

Colonies of molds and yeasts should be apparent within 5 days of incubation. Colonies of yeast appear pink due to the uptake of Rose Bengal. Report the results as colony forming units per gram or milliliter of sample.

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. and 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, (ed.). 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 (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association. Washington, D.C.

Packaging

DRBC Agar	500 g	0587-17

Decarboxylase Differential Media

Bacto® Decarboxylase Base Moeller · Bacto Decarboxylase Medium Base · Bacto Lysine Decarboxylase Broth

Intended Use

Bacto Decarboxylase Base Moeller is a basal medium which, with added lysine, arginine, ornithine, or another amino acid, is used for differentiating bacteria based on their ability to decarboxylate amino acids.

Bacto Decarboxylase Medium Base, with added lysine, arginine, or ornithine, is used for differentiating bacteria based on amino acid decarboxylation.

Bacto Lysine Decarboxylase Broth is used for differentiating microorganisms based on lysine decarboxylation.

Also Known As

Decarboxylase Base Moeller is also referred to as Moeller Decarboxylase Broth Base. Decarboxylase Medium Base is also known as Decarboxylase Medium Base, Falkow or Decarboxylase Basal Medium.

Summary and Explanation

Moeller [1,2,3] described the amino acid decarboxylase test for distinguishing between various microorganisms. He determined the usefulness of using this enzyme system in the differentiation of the *Enterobacteriaceae.*[4,5] The production of lysine, arginine, ornithine, and glutamic acid decarboxylase by various members of this family provided a useful adjunct to other biochemical tests used for the speciation and identification of the *Enterobacteriaceae.*

Carlquist[6] developed a medium using the lysine decarboxylase reaction to differentiate *Salmonella arizonae (Arizona)* from *Citrobacter* (Bethesda-Ballerup biotype). Falkow[7] obtained valid and reliable results with a lysine decarboxylase medium he developed to differentiate and identify *Salmonella* and *Shigella.* Although his modification of the Moeller formula was originally described as a lysine medium only, further study by Falkow and then by Ewing, Davis and Edwards,[8] substantiated the use of the medium for ornithine and arginine decarboxylase reactions as well.

Ewing, Davis and Edwards[8] compared the Falkow decarboxylase medium base to the Moeller medium and reported that, although the two methods compared favorably in most cases, the Moeller medium was found to be more reliable for cultures of *Klebsiella* and *Enterobacter.* They concluded that the Moeller method should be regarded as the standard or reference method, although the Falkow formula is suitable for determining decarboxylase reactions for most members of the *Enterobacteriaceae* except for *Klebsiella* and *Enterobacter.* The Moeller medium is also particularly useful in the identification of *Aeromonas*, *Plesiomonas*, *Vibrio* spp., and nonfermentative gram- negative bacilli.[9]

Decarboxylase Base Moeller conforms with the Moeller formulation while Decarboxylase Medium Base is prepared according to the formula described by Falkow. Lysine Decarboxylase Broth is the Falkow medium with L-Lysine added in 0.5% concentration.

Decarboxylase tests are important in the differentiation and identification of a wide variety of microorganisms and are outlined in numerous standard methods.[10-13]

Principles of the Procedure

Decarboxylase Base Moeller, Decarboxylase Medium Base and Lysine Decarboxylase Broth consist of Bacto Peptone and Beef Extract which supply carbon and nitrogen. Dextrose is a fermentable carbohydrate. Yeast Extract provides vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon. As applicable, Brom Cresol Purple and Cresol Red are pH indicators. The Pyridoxal is an enzyme cofactor for the amino acid decarboxylase. The amino acids lysine, ornithine, and arginine are added to the basal media to detect the production of the enzymes specific for these substrates.

When the media are inoculated with bacteria that are able to ferment the dextrose, acids are produced that lower the pH and change the indicator from purple to yellow. If the bacteria produce the appropriate decarboxylase, the production of amines raises the pH of the medium causing the indicator to change from yellow to a light or deep purple. Decarboxylation of lysine yields cadaverine, while decarboxylation of ornithine yields putrescine. Arginine is first hydrolyzed to ornithine and then decarboxylated to putrescine. If decarboxylation does not occur the medium remains acidic (yellow). Control tubes of basal media, that do not contain an amino acid, should be inoculated to verify reactions.

To obtain proper reactions, inoculated tubes must be protected from the air. This is done to avoid false alkalinization at the surface of the medium, which could cause a decarboxylase negative bacteria to appear to be positive. This can be done by overlaying a medium with sterile mineral oil as suggested by Ewing, Davis and Edwards.[8]

Formula

Decarboxylase Base Moeller

Formula Per Liter

Bacto Peptone	5 g
Bacto Beef Extract	5 g
Bacto Dextrose	0.5 g
Bacto Brom Cresol Purple	0.01 g
Cresol Red	0.005 g
Pyridoxal	0.005 g

Final pH 6.0 ± 0.2 at 25°C

Decarboxylase Medium Base

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	3 g
Bacto Dextrose	1 g
Bacto Brom Cresol Purple	0.02 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Decarboxylase Base Moeller

Dehydrated Appearance:	Light to medium tan, homogeneous, free-flowing.
Solution:	1.05% solution, soluble in distilled or deionized water on warming.
Prepared Tubes:	Yellowish-red, slightly opalescent.
pH at 25°C:	6.0 ± 0.2

Decarboxylase Medium Base

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	0.9% solution, soluble in distilled or deionized water on warming.
Prepared Tubes:	Purple, clear.
pH at 25°C:	6.8 ± 0.2

Lysine Decarboxylase Base

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	1.4% solution, soluble in distilled or deionized water on warming.
Prepared Tubes:	Purple, clear w/o significant precipitate.
pH at 25°C:	6.8 ± 0.2

Cultural Response

Prepare media per label directions. Where necessary add appropriate amounts of amino acids to be tested. Inoculate with approx. 1,000 CFUs of test organisms and overlay test tubes with sterile mineral oil. Incubate at 35 ± 2°C for 18-48 hours. Purple color indicates a positive decarboxylase reaction. Yellow color indicates a negative decarboxylase reaction.

Decarboxylase Base Moeller

ORGANISM	ATCC®	GROWTH	REACTION w/o LYSINE	REACTION w/ LYSINE
Escherichia coli	25922*	good	yellow (–)	purple (+)
Shigella flexneri	12022*	good	yellow (–)	yellow (–)

Decarboxylase Medium Base

ORGANISM	ATCC®	GROWTH	REACTION LYSINE	REACTION ORNITHINE	REACTION ARGININE
Salmonella typhimurium	14028*	good	purple (+)	purple (+)	purple (+)
Proteus vulgaris	13315*		yellow (–)	yellow (–)	yellow (–)

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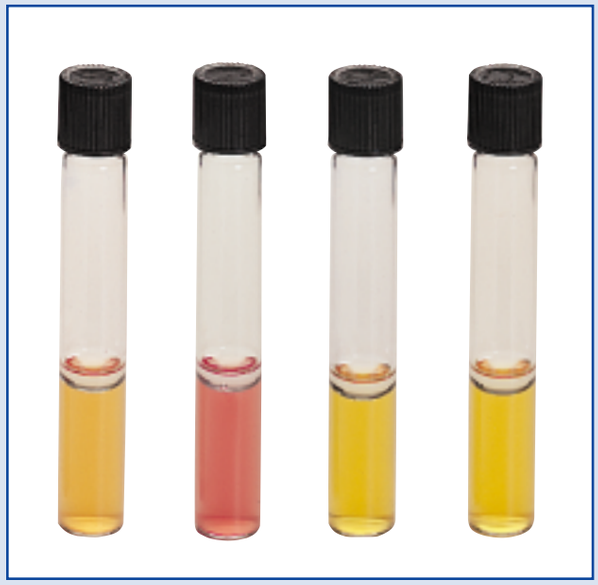
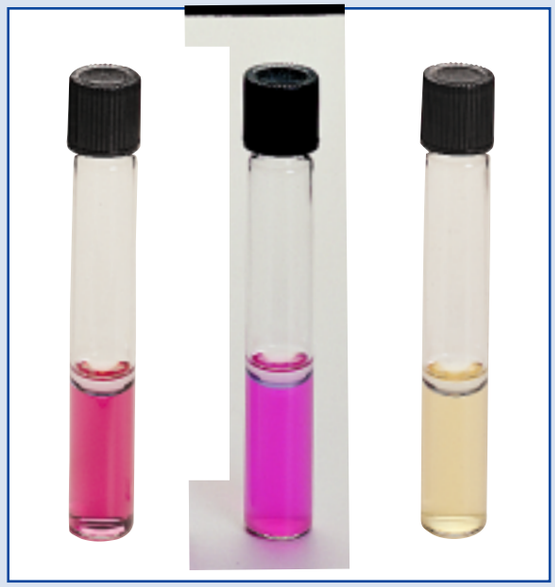


Escherichia coli ATCC® 25922 | *Escherichia coli* ATCC® 25922 w/ Lysine | *Shigella flexneri* ATCC® 12022 | *Shigella flexneri* ATCC® 12022 w/ Lysine

Decarboxylase Base Moeller



Uninoculated tube | *Salmonella typhimurium* ATCC® 14028 | *Proteus vulgaris* ATCC® 13315

Decarboxylase Medium Base

Lysine Decarboxylase Broth

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	3 g
Bacto Dextrose	1 g
L-Lysine	5 g
Bacto Brom Cresol Purple	0.02 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated media below 30°C. The dehydrated media is very hygroscopic. Keep containers tightly closed.

Expiration Date

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

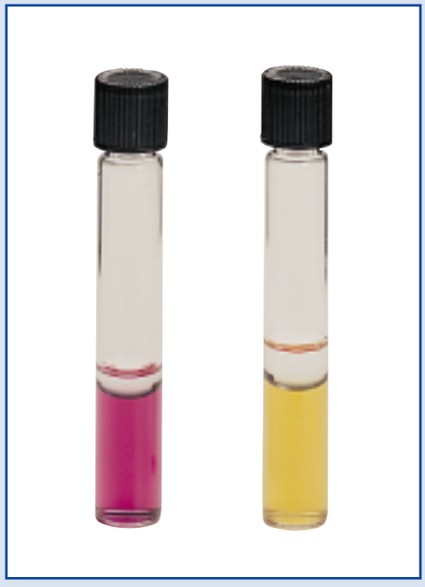
User Quality Control cont.

Lysine Decarboxylase Base

ORGANISM	ATCC®	GROWTH	REACTION
Escherichia coli	25922*	good	purple (+)
Proteus vulgaris	13315*	good	yellow (–)

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Escherichia coli ATCC® 25922 *Proteus vulgaris* ATCC® 13315

Lysine Decarboxylase Broth

Procedure

Materials Provided

Decarboxylase Base Moeller
Decarboxylase Medium Base
Lysine Decarboxylase Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (37°C)
Wire loops (bacteriological)
L-lysine, L-arginine, L-ornithine, or other L-amino acids (to be added to either Decarboxylase Base Moeller or Decarboxylase Medium Base)
Sterile mineral oil
1N NaOH

Methods of Preparation

Decarboxylase Base Moeller

1. Suspend 10.5 grams in 1 liter distilled or deionized water and heat to dissolve completely.
2. Add 10 grams L-amino acid (or 20 grams DL-amino acid) and agitate to dissolve completely. When adding ornithine which is highly acidic, adjust the pH with NaOH (approximately 4.6 ml 1 N NaOH per liter) prior to sterilizing.
3. Dispense 5 ml amounts into screw capped test tubes.
4. Autoclave at 121°C for 10 minutes.

Decarboxylase Medium Base

1. Suspend 9 grams in 1 liter distilled or deionized water and warm to dissolve completely.
2. Add 5 grams L-amino acid (or 10 grams DL-amino acid) and warm to dissolve completely. Adjust the pH with NaOH (if necessary) prior to sterilizing.
3. Dispense 5 ml amounts into screw capped test tubes.
4. Autoclave at 121°C for 15 minutes.

Lysine Decarboxylase Broth

1. Suspend 14 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense 5 ml amounts into screw capped test tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Only pure cultures of enteric bacteria taken from purification plates or agar slants are to be used for biochemical tests. A presumptive identification of the bacteria under investigation should be made on the basis of morphological and cultural characteristics prior to biochemical testing.

Test Procedure

1. Inoculate the prepared tubes with a 24 hour pure culture using a bacteriological loop. A control tube should also be inoculated.
2. Aseptically overlay all inoculated tubes, including the control tube, with 4-5 mm sterile mineral oil.
3. Incubate tubes at 35 ± 2°C for up to 4 days. Tubes must be read daily after 24 hours incubation. Observe for a change in color from purple to yellow to purple.

Results

See appropriate reference for the expected decarboxylase reactions of the *Enterobacteriaceae* and other organisms.[14]

Limitations of the Procedure

1. Biochemical characteristics of the *Enterobacteriaceae* serve to confirm presumptive identification based on cultural, morphological, and/or serological findings. Therefore, biochemical testing should be attempted on pure culture isolates only and subsequent to differential determinations.
2. The decarboxylase reactions are part of a total biochemical profile for members of the *Enterobacteriaceae* and related organisms. Results obtained from these reactions, therefore, can be considered indicative of a given genus or species. However, conclusive and final identification of these organisms cannot be made solely on the basis of the decarboxylase reactions.
3. If layers of yellow and purple appear after incubation, shake the test tube gently before attempting to interpret results.
4. If a reaction is difficult to interpret, compare the tube in question to an uninoculated control tube. Any trace of purple after 24 hours of incubation is a positive test.
5. A gray color may indicate reduction of the indicator. Additional indicator may be added before the results are interpreted.[12]
6. *Salmonella gallinarum* gives a delayed positive ornithine decarboxylase reaction, requiring 5-6 days incubation.[3] Many strains of *E. coli*, including those that ferment adonitol, may exhibit a delayed reaction.[3]
7. Decarboxylase Medium Base is not satisfactory for the determination of lysine decarboxylase activity with the two genera *Klebsiella* and *Enterobacter.*
8. The lysine decarboxylase activity in *Salmonella* is used to differentiate this group from *Citrobacter freundii*. *Salmonella paratyphi A,* however, gives an atypical negative reaction (yellow color of medium) in 24 hours when Decarboxylase Medium Base is used.[15]

References

1. **Moeller, V.** 1954. Activity determination of amino acid decarboxylases in *Enterobacteriaceae*. Acta Pathol. Microbiol. Scand. **34**: 102-111.
2. **Moeller, V.** 1954. Distribution of amino acid decarboxylases in *Enterobacteriaceae*. Acta Pathol. Microbiol. Scand. **34**: 259-277.
3. **Moeller, V.** 1955. Simplified tests of some amino acid decarboxylases for arginine dihydrolase system. Acta Pathol. Microbiol. Scand. **36**: 158-172.
4. **Gale, E. F.** 1940. The production of amines by bacteria. Biochem. J. **34**: 392, 583, 846.
5. **Gale, E. F.** 1941. Production of amines by bacteria. 4. The decarboxylation of amino-acids by organisms of the groups *Clostridium* and *Proteus*. Biochem. J. **35**: 66-79.
6. **Carlquist, P. R.** 1956. A biochemical test for separating paracolon groups. J. Bacteriol. **71**: 339-341.
7. **Falkow, S.** 1958. Activity of lysine decarboxylase as an aid in the identification of *Salmonella* and *Shigella*. Am. J. Clin. Pathol. **29**: 598.
8. **Ewing, W. H., B. R. Davis, and P. R. Edwards.** 1960. The decarboxylase reaction of *Enterobacteriaceae* and their value in taxonomy. Publ. Health Lab. **18**: 77-83.
9. **Baron, E J., L R. Peterson, and S. M. Finegold (eds.).** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
10. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
11. **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.
12. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
13. **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.
14. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken.** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
15. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Decarboxylase Base Moeller	100 g	0890-15
	500 g	0890-17
Decarboxylase Medium Base	500 g	0872-17
	10 kg	0872-08
Lysine Decarboxylase Broth	100 g	0215-15
	500 g	0215-17
	10 kg	0215-08

Bacto® Demi-Fraser Broth Base
Bacto Fraser Broth Supplement

Intended Use

Bacto Demi-Fraser Broth Base is used with Bacto Fraser Broth Supplement in selectively and differentially enriching *Listeria* from foods.

Summary and Explanation

Fraser Broth Base and Fraser Broth Supplement are based on the Fraser Broth formulation of Fraser and Sperber.[1] The medium is used in the rapid detection of *Listeria* from food and environmental samples. Demi-Fraser Broth Base is a modification of Fraser Broth Base in which the nalidixic acid and acriflavine concentrations have been reduced to 10 mg/l and 12.5 mg/l respectively, in accordance with AFNOR guidelines.[2]

Principles of the Procedure

Tryptose, Beef Extract and Yeast Extract provide carbon and nitrogen sources and the cofactors required for good growth of *Listeria*. Sodium Phosphate and Potassium Phosphate buffer the medium. Selectivity is provided by Lithium Chloride, Nalidixic Acid and Acriflavine. The high Sodium Chloride concentration of the medium inhibits growth of enterococci.

All *Listeria* species hydrolyze esculin, as evidenced by a blackening of the medium. This blackening results from the formation of 6,7-dihydroxycoumarin, which reacts with ferric ions.[1] Ferric ions are added to the final medium as Ferric Ammonium Citrate in Fraser Broth Supplement.

Formula

Demi-Fraser Broth Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Sodium Chloride	20 g
Sodium Phosphate, Dibasic	9.6 g
Potassium Phosphate, Monobasic	1.35 g
Esculin	1 g
Nalidixic Acid	0.01 g
Acriflavine HCl	0.0125 g
Lithium Chloride	3 g

Final pH 7.2 ± 0.2 at 25°C

Fraser Broth Supplement

Ingredients per 10 ml vial

Ferric Ammonium Citrate	0.5 g

One vial is added to one liter of basal medium

Precautions

1. For Laboratory Use.
2. **Demi-Fraser Broth Base: HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Fraser Broth Supplement: **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

User Quality Control

Identity Specifications

Demi-Fraser Broth Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in distilled or deionized water. Solution is medium amber, clear to slightly opalescent, may have a fine precipitate.
Prepared Medium:	Medium amber, very slightly to slightly opalescent, may have a slight precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.2 ± 0.2

Fraser Broth Supplement

Solution Appearance: Dark brown solution.

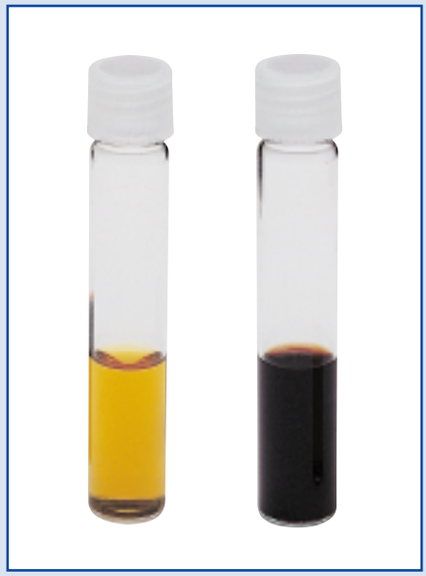
Cultural Response

Prepare Demi-Fraser Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH/APPEARANCE
Escherichia coli	25922*	1,000-2,000	inhibited
Listeria monocytogenes	19114	100-1,000	good growth/blackening of the medium
Enterococcus faecalis	29212*	1,000-2,000	markedly to completely inhibited

The cultures listed above are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube — *Listeria monocytogenes* ATCC® 19114

3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Fraser Broth Supplement at 2-8°C.

Store the prepared medium at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Demi-Fraser Broth Base
Fraser Broth Supplement

Materials Required But Not Provided

Glassware
Autoclave
Fraser Broth
Oxford Medium
PALCAM Medium
Sterile tubes with closures

Method of Preparation

1. Dissolve 55 grams of Demi-Fraser Broth Base in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.
3. Aseptically add 10 ml Fraser Broth Supplement. Mix well.

Sample Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[2]

1. Pre-enrich the sample in Demi-Fraser Broth. Incubate for 18-24 hours at 35 ± 2°C. Subculture onto Oxford Medium or PALCAM Medium.
2. Transfer 0.1 ml of the pre-enrichment culture into 10 ml of Fraser Broth and incubate for 48 hours at 37°C. Subculture onto Oxford Medium or PALCAM Medium after 18-24 hours and again after 42-48 hours of incubation.
3. Examine Oxford Medium or PALCAM Medium plates for the appearance of presumptive *Listeria* colonies.
4. Confirm the identity of all presumptive *Listeria* by biochemical and/or serological testing.

Results

The presence of *Listeria* is presumptively indicated by the blackening of Demi-Fraser Broth after incubation for 24-48 hours at 35°C. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/serological identification.

References

1. **Fraser, J., and W. Sperber.** 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. Journal of Food Protection **51**:762- 765.
2. **L'association française de normalisation (AFNOR).** 1993. Food Microbiology- Detection of *Listeria monocytogenes*-Routine Method, V 08-055. AFNOR, Paris, France.

Packaging

Demi-Fraser Broth Base	500 g	0653-17-0
	10 kg	0653-07-0
Fraser Broth Supplement	6 x 10 ml	0211-60-2

Bacto® Desoxycholate Agar

Intended Use

Bacto Desoxycholate Agar is used for isolating and differentiating gram-negative enteric bacilli.

Also Known As

Deoxycholate Agar (Sodium Deoxycholate Agar)

NOTE: Alternate spelling[1] - Deoxy-.

Summary and Explanation

Desoxycholate Agar as formulated by Leifson[2] demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora. The medium was an improvement over other media of the time because the chemicals, citrates and sodium desoxycholate, in specified amounts, worked well as inhibitors. This medium has been used to screen for *Salmonella* sp. and *Shigella* sp. from clinical specimens.[3]

Principles of the Procedure

Bacto Peptone provides nitrogen and carbon for general growth requirements. Lactose is the fermentable carbohydrate. Sodium chloride and dipotassium phosphate maintain the osmotic balance of the medium. Sodium desoxycholate, ferric citrate and sodium citrate inhibit growth of gram-positive bacteria. Neutral red is a pH indicator. Bacto® Agar is a solidifying agent.

Differentiation of enteric bacilli is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of neutral red, form red colonies. Bacteria that do not ferment lactose form colorless colonies. The majority of normal intestinal bacteria ferment lactose (red colonies) while *Salmonella* and *Shigella* species do not ferment lactose (colorless colonies).

Formula

Desoxycholate Agar

Formula Per Liter

Bacto Peptone	10 g
Bacto Lactose	10 g

Sodium Desoxycholate	1 g
Sodium Chloride	5 g
Dipotassium Phosphate	2 g
Ferric Citrate	1 g
Sodium Citrate	1 g
Bacto Agar	15 g
Neutral Red	0.03 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Desoxycholate Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Bunsen burner or heating plate
Incubator (35°C)
Petri dishes

Method of Preparation

1. Suspend 45 grams in 1 liter distilled or deionized water.
2. Boil 1 minute with frequent, careful agitation to dissolve completely. Avoid overheating.
3. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For a complete discussion on the isolation of enteric bacilli, refer to appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for results.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 269-275, vol 1. Williams & Wilkins, Baltimore, MD.
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**:581-599.

User Quality Control

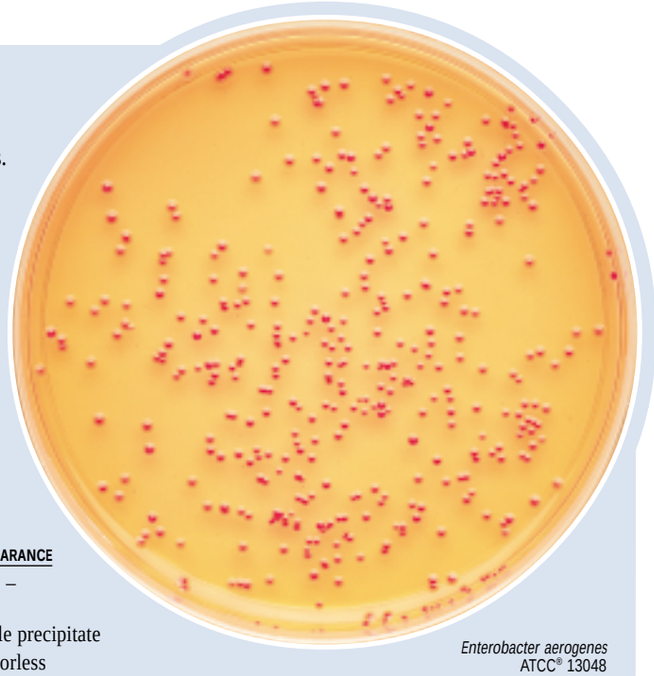
Identity Specifications

Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous.
Solution:	4.5% solution, soluble in distilled or deionized water on boiling. Solution is reddish orange, very slightly to slightly opalescent with no significant precipitate.
Prepared Medium:	Orange, slightly opalescent.
Reaction of 4.5% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Desoxycholate Agar per label directions. Inoculate using the pour plate method and incubate plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition	–
Escherichia coli	25922*	30-300	good	pink w/bile precipitate
Salmonella typhimurium	14028*	30-300	good	colorless



Enterobacter aerogenes
ATCC® 13048

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

3. **Balows, A., W. J. Mausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadony (ed.)** 1991. Manual of clinical microbiology. 5th ed. American Society for Microbiology, Washington, D.C.

Packaging

Desoxycholate Agar	500 g	0273-17

Bacto® Desoxycholate Citrate Agar

Intended Use

Bacto Desoxycholate Citrate Agar is used for isolating enteric bacilli, particularly *Salmonella* and many *Shigella* species.

Also Known As

NOTE: Deoxy-; alternate spelling.[1]

Summary and Explanation

Desoxycholate Citrate Agar is a modification of Desoxycholate Agar formulated by Leifson.[2] His original medium demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using citrates and sodium desoxycholate in specified amounts as inhibitors to gram positive bacteria.

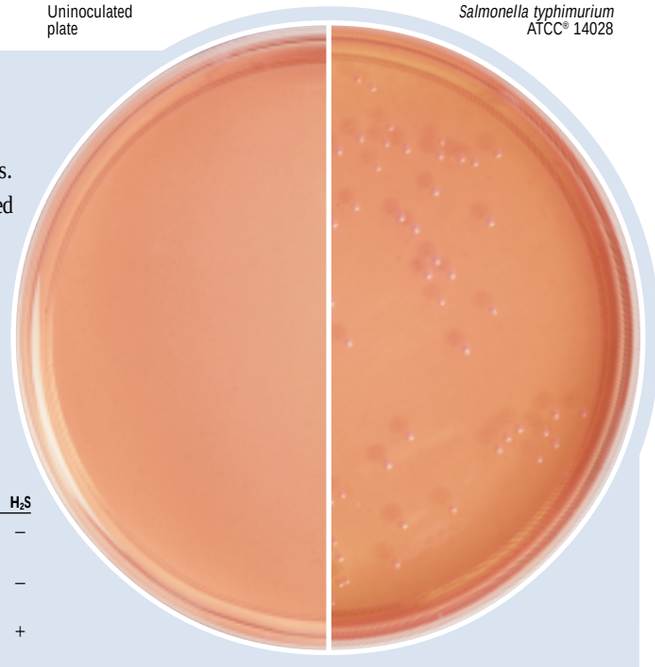
Leifson modified his original medium by increasing the concentration of sodium citrate and sodium desoxycholate and found Desoxycholate Citrate Agar reliable for isolating many *Salmonella* and *Shigella* species.[2]

Desoxycholate Citrate Agar effectively isolates intestinal pathogens (*Salmonella* and *Shigella* species) by inhibiting coliforms and many *Proteus* species.[1] This medium is widely used by clinical laboratories.[3]

Principles of the Procedure

Infusion from Meat is a source of carbon and nitrogen. This ingredient is used because the inhibition of coliforms produced is greater than when an extract or simple peptone is used.[2] Desoxycholate Citrate Agar contains Proteose Peptone No. 3 as a source of carbon, nitrogen, vitamins and minerals. Lactose is a carbohydrate. Sodium Citrate and Sodium Desoxycholate inhibit gram positive bacteria, coliforms and *Proteus* species. Ferric Ammonium Citrate aids in the detection of H_2S producing bacteria. Neutral Red is a pH indicator. Bacto Agar is a solidifying agent.

In the presence of neutral red, bacteria that ferment lactose produce acid and form red colonies. Bacteria that do not ferment lactose form colorless colonies. If the bacteria produce H_2S, the colonies will have black centers. The majority of normal intestinal bacteria ferment lactose and do not produce H_2S (red colonies without black centers). *Salmonella* and *Shigella* spp. do not ferment lactose but *Salmonella* may produce H_2S (colorless colonies with or without black centers). Lactose-fermenting colonies may have a zone of precipitation around them caused by the precipitation of desoxycholate in the presence of acid.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish-beige, free-flowing, homogeneous.
Solution:	7.0% solution, soluble in distilled or deionized water on boiling. Solution is orange-red, very slightly to slightly opalescent.
Prepared Medium:	Orange-red, slightly opalescent.
Reaction of 7.0% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Prepare Desoxycholate Citrate Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	H_2S
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition	–	–
Escherichia coli	25922*	100-1,000	partial inhibition	pink with bile precipitate	–
Salmonella typhimurium	14028*	100-1,000	fair to good	colorless	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

Desoxycholate Citrate Agar

Formula Per Liter

Meat, Infusion from	330 g
Bacto Proteose Peptone No. 3	10 g
Bacto Lactose	10 g
Sodium Citrate	20 g
Ferric Ammonium Citrate	2 g
Sodium Desoxycholate	5 g
Bacto Agar	13.5 g
Neutral Red	0.02 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Desoxycholate Citrate Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Incubator (35°C)

Method of Preparation

1. Suspend 70 grams in 1 liter distilled or deionized water.
2. Heat and boil briefly with frequent, careful agitation to dissolve completely. Avoid overheating.
3. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

1. Inoculate specimen directly onto surface of medium.
2. Incubate plates at 35 ± 2°C for 18-24 hours. Plates can be incubated for an additional 24 hours if no lactose fermenters are observed.

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.

Limitations of the Procedure

1. Coliform strains may be encountered that will grow on this medium, making it difficult to detect pathogens.
2. Heavy inocula should be distributed over the entire surface of the medium prevent complete masking of pathogens by coliform organisms.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 269-275. Williams & Wilkins, Baltimore, MD.
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**: 581-599.
3. **Farmer III, J. J., and M. T. Kelly.** 1991. *Enterobacteriaceae.* p. 360-383. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Packaging

Desoxycholate Citrate Agar	500 g	0274-17

Bacto® Desoxycholate Lactose Agar

Intended Use

Bacto Desoxycholate Lactose Agar is used for isolating and differentiating gram-negative enteric bacilli and for enumerating coliforms from water, wastewater, milk and dairy products.

Also Known As

NOTE: Deoxy-; alternate spelling.[1]

Summary and Explanation

Desoxycholate Lactose Agar is a modification of Desoxycholate Agar formulated by Leifson.[2] His original medium demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using citrates and sodium desoxycholate in specified amounts as inhibitors to gram-positive bacteria.

Standard Methods manuals for dairy[3] and water[4] specified a modification of Desoxycholate Agar to contain less sodium desoxycholate and, accordingly, be less inhibitory to gram-positive bacteria. This formulation, known as Desoxycholate Lactose Agar, was used in pour plate procedures for isolation and enumeration of coliforms in milk, water and other specimens.

Principles of the Procedure

Bacto Peptone provides nitrogen and carbon for general growth requirements. Lactose is a fermentable carbohydrate. Sodium

Chloride maintains the osmotic balance of the medium. Sodium Desoxycholate and Sodium Citrate inhibit growth of gram-positive bacteria. Neutral Red is a pH indicator. Bacto Agar is a solidifying agent.

Differentiation of enteric bacilli is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of neutral red, form red colonies. Bacteria that do not ferment lactose form colorless colonies. The majority of normal intestinal bacteria ferment lactose (red colonies) while *Salmonella* and *Shigella* species do not ferment lactose (colorless colonies).

Formula

Bacto Desoxycholate Lactose Agar

Formula Per Liter

Bacto Proteose Peptone	10 g
Bacto Lactose	10 g
Sodium Desoxycholate	0.5 g
Sodium Chloride	5 g
Sodium Citrate	2 g
Bacto Agar	15 g
Neutral Red	0.03 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Bacto Desoxycholate Lactose Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Bunsen burner or heating plate
Incubator(35°C)
Petri dishes

Method of Preparation

1. Suspend 42.5 grams in 1 liter distilled or deionized water.
2. Boil 1 minute with frequent, careful agitation to dissolve completely. Avoid overheating.
3. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

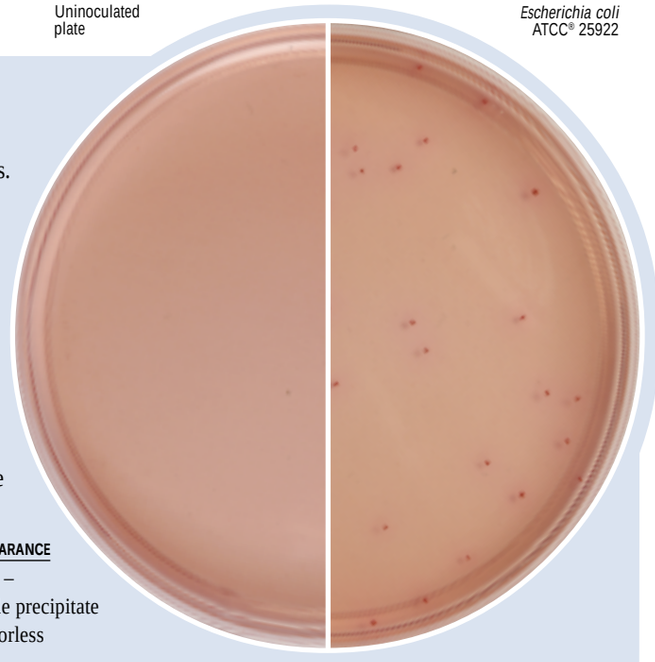
Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous.
Solution:	4.25% solution, soluble in distilled or deionized water on boiling. Solution is pinkish-red, very slightly to slightly opalescent.
Prepared Medium:	Pinkish-red, very slightly to slightly opalescent.
Reaction of 4.25% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1000-2,000	markedly inhibited	–
Escherichia coli	25922*	30-300	good	pink w/bile precipitate
Salmonella typhimurium	14028*	30-300	good	colorless

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 269-275, vol 1. Williams & Wilkins, Baltimore, MD.
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**:581-599.
3. **American Public Health Association.** 1960. Standard methods for the examination of dairy products microbiological and chemical, 11th ed. American Public Health Association, Washington, D.C.
4. **American Public Health Association.** 1960. Standard methods for the examination of water and wastewater, 11th ed. American Public Health Association, Washington, D.C.

Packaging

Desoxycholate Lactose Agar	500 g	0420-17

Bacto® Dextrose Agar
Bacto Dextrose Broth

Intended Use

Bacto Dextrose Agar is used for cultivating a wide variety of microorganisms with or without added blood.

Bacto Dextrose Broth is used for cultivating fastidious microorganisms and for detecting gas from enteric bacilli.

Summary and Explanation

In 1932, Norton[1] recommended a basal medium containing 0.5-1% dextrose with approximately 5% defibrinated blood for the isolation of many fastidious bacteria, including *Haemophilus* and *Neisseria*. Dextrose is an energy source used by many organisms. The high concentration of this ingredient makes Dextrose Agar a suitable medium for the production of early, abundant organism growth and shortening the lag periods of older cultures. Because of the increased dextrose content, Dextrose Agar is not suitable for observation of hemolysis when supplemented with 5% sheep, rabbit or horse blood.

Dextrose Broth is a highly nutritious broth suitable for the isolation of fastidious organisms and specimens containing a low inoculum. The addition of 0.1-0.2% agar to Dextrose Broth facilitates anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment.[2] The low agar concentration provides suitable conditions for both aerobic growth in the clear upper zone and for microaerophilic and anaerobic growth in the lower, flocculent agar zones.

Dextrose Agar and Dextrose Broth are specified in the Compendium of Methods for the Microbiological Examination of Foods.[3]

Principles of the Procedure

Beef Extract and Tryptose provide nitrogen, amino acids and vitamins. Dextrose is a carbon source, and the increased concentration is a distinguishing characteristic of this medium from other formulations used

User Quality Control

Identity Specifications

Dextrose Agar

Dehydrated Appearance:	Medium beige, homogeneous, free-flowing.
Solution:	4.3% solution, soluble in distilled or deionized water on boiling; medium amber, very slightly to slightly opalescent.
Prepared Medium:	Plain - Medium amber, slightly opalescent without significant precipitate. With blood - Cherry-red, opaque.
Reaction of 4.3% Solution at 25°C	pH 7.3 ± 0.2

Dextrose Broth

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
Solution:	2.3% solution, soluble in distilled or deionized water; light to medium amber, clear without significant precipitate.
Prepared Medium:	Light to medium amber.
Reaction of 2.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Dextrose Agar

Prepare Dextrose Agar per label directions with and without sterile defibrinated sheep blood. Inoculate and incubate at 35 ± 2°C under proper atmospheric conditions for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o BLOOD	GROWTH w/5% SHEEP BLOOD
Neisseria meningitidis	13090*	100-1,000	poor	good
Staphylococcus aureus	25923*	100-1,000	good	good
Streptococcus pyogenes	19615*	100-1,000	good	good

Dextrose Broth

Prepare Dextrose Broth per label directions with and without 0.1% Bacto Agar; dispense into tubes containing fermentation vials. Inoculate and incubate at 35 ± 2°C under proper atmospheric conditions. Read growth and gas production at 15-24 and 40-48 hours.

ORGANISM	ATCC®	GROWTH	CFU	GAS PRODUCTION	GROWTH w/1% AGAR
Escherichia coli	25922*	good	100-1,000	+	good
Neisseria meningitidis	13090*	good	100-1,000	–	good
Streptococcus pyogenes	19615*	good	100-1,000	–	good
Staphylococcus aureus	25923*	good	100-1,000	–	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

as blood agar bases. Bacto Agar is a solidifying agent.

Supplementation with 5% blood provides additional growth factors for fastidious microorganisms.

Formula

Dextrose Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Tryptose	10 g
Bacto Dextrose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Dextrose Broth

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Tryptose	10 g
Bacto Dextrose	5 g
Sodium Chloride	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Dextrose Agar
Dextrose Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood (optional)
Sterile Petri dishes

Method of Preparation

Dextrose Agar

1. Suspend 43 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense into sterile Petri dishes or as desired.

Dextrose Broth

1. Suspend 23 grams in 1 liter distilled or deionized water.
2. Dissolve in distilled or deionized water.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into tubes.

Specimen Collection and Preparation

Specimens are obtained and processed according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion on microorganism isolation and identification, refer to appropriate references.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Norton.** 1932. Bacteriology of pus. J. Lab. Clin. Med. **17**:558-565.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 802-804. Williams & Wilkins, Baltimore, MD.
3. **Vanderzant, C. and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Dextrose Agar	500 g	0067-17
Dextrose Broth	500 g	0063-17

Bacto® Dextrose Starch Agar

Intended Use

Bacto Dextrose Starch Agar is used for cultivating pure cultures of *Neisseria gonorrhoeae* and other fastidious microorganisms.

Summary and Explanation

Dextrose Starch Agar is recommended as a complete solid medium for the propagation of pure cultures of *Neisseria gonorrhoeae*. This highly nutritious medium without additives will also support excellent growth of *N. meningitidis*, *Streptococcus pneumoniae* and *S. pyogenes*. Dextrose Starch Agar, in half concentration, is recommended as a Stock Culture Agar for the maintenance of *N. gonorrhoeae*, *N. meningitidis* and other organisms not capable

of hydrolyzing starch. This medium cannot be used to maintain stock cultures of organisms capable of splitting starch; acid production from starch will create an unsatisfactory environment.

Dextrose Starch Agar was used by Wilkins, Lewis and Barbiers[1] in an agar dilution procedure to test the activity of antibiotics against *Neisseria* species.

Principles of the Procedure

Proteose Peptone No. 3 and Gelatin provide the nitrogen, vitamins and amino acids in Dextrose Starch Agar. Soluble Starch improves growth response. Dextrose is a carbon source. Sodium chloride maintains the osmotic balance of the medium, and disodium phosphate is a buffering agent. Bacto Agar is the solidifying agent.

Formula

Dextrose Starch Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	15 g
Bacto Dextrose	2 g
Bacto Soluble Starch	10 g
Sodium Chloride	5 g
Disodium Phosphate	3 g
Bacto Gelatin	20 g
Bacto Agar	10 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6.5 % solution, soluble in distilled or deionized water on boiling; light amber, opalescent with a precipitate.
Prepared Medium:	Light amber, opalescent with a precipitate.
Reaction of 6.5% Solution at 25°C	pH 7.3 ± 0.2

Cultural Response

Prepared Dextrose Starch Agar per label directions. Incubate inoculated medium at 35 ± 2°C for 18-48 hours under 5-10% CO_2.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria gonorrhoeae	CDC 98*	100-1,000	good
Neisseria meningitidis	13090 98*	100-1,000	good
Streptococcus pyogenes	19615 98*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Material Provided

Dextrose Starch Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 65 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion of the isolation and identification of *N. gonorrhoeae* and other fastidious pathogens, refer to the procedures described in Clinical Microbiology Procedures Handbook[2] and Manual of Clinical Microbiology.[3]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. This medium is not recommended for isolation of gonococci from mixed cultures.

References

1. **Wilkins, Lewis, and Barbiers.** 1956. Antibiot. Chemother. **6**:149.
2. **Isenberg, 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. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Dextrose Starch Agar	500 g	0066-17
	10 kg	0066-08

Bacto® Dextrose Tryptone Agar

Intended Use

Bacto Dextrose Tryptone Agar is used for cultivating thermophilic "flat-sour" microorganisms associated with food spoilage.

Summary and Explanation

In the 1930's, the National Canners Association specified the use of Dextrose Tryptone Agar for isolating "flat sour" organisms from food products.[1] "Flat sour" spoilage of canned foods is caused by *Bacillus coagulans* (*Bacillus thermoacidurans*). Bacterial growth results in a 0.3-0.5 drop in pH, while the ends of the can remain flat. *B. coagulans* is a soil microorganism that can be found in canned tomato products and dairy products. Conditions favorable for multiplication of the bacterium can result in spoilage of the food product.[2]

Dextrose Tryptone Agar can also be used to isolate other food spoilage bacteria: mesophilic aerobic spore formers in the genera *Bacillus* and *Sporolactobacillus* and thermophilic flat sour spore formers such as *B. stearothermophilus*.[2]

Principles of the Procedure

Dextrose Tryptone Agar contains Tryptone to provide carbon and nitrogen sources for general growth requirements. Dextrose is the carbohydrate source. Brom Cresol Purple is the pH indicator. Bacto Agar is the solidifying agent.

Formula

Bacto Dextrose Tryptone Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Dextrose	5 g
Bacto Agar	15 g
Bacto Brom Cresol Purple	0.04 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Bacto Dextrose Tryptone Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Petri dishes
Incubator

User Quality Control

Identity Specifications

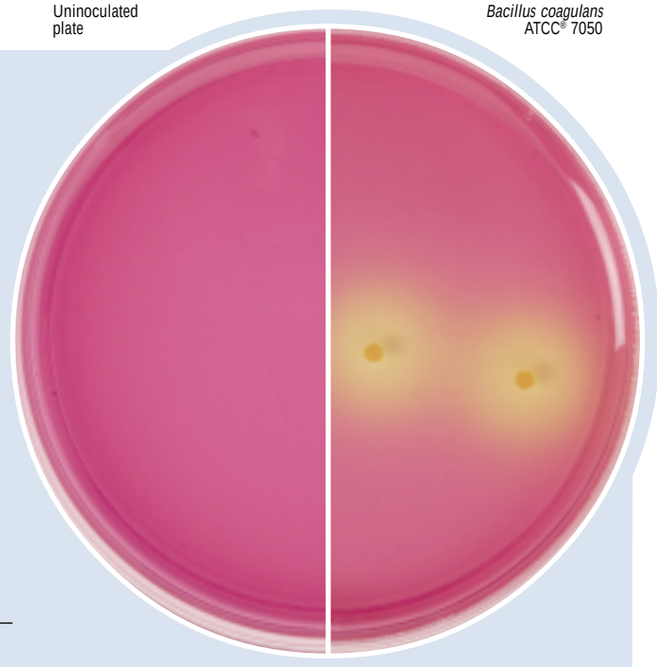
Dehydrated Appearance:	Light, greenish-beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in distilled or deionized water on boiling; purple, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Purple, slightly opalescent without significant precipitate.
Reaction of 3.0% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Dextrose Tryptone Agar per label directions. Inoculate plates and incubate at 55°C for 36-48 hours. Examine cultures for growth. A change in the color of the medium from purple to yellow indicates dextrose fermentation.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Bacillus coagulans	7050	100-1,000	good	yellow
Bacillus stearothermophilus	7953	100-1,000	good	yellow

The cultures listed are the minimum that should be used for performance testing.



Method of Preparation

1. Suspend 30 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **National Canners Association**. 1933. Bacterial standards for sugar.
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Dextrose Tryptone Agar	500 g	0080-17

Bacto® Differential Reinforced Clostridial Agar

Intended Use

Bacto Differential Reinforced Clostridial Agar is used for enumerating and cultivating sulfite-reducing clostridia.

Also Known As

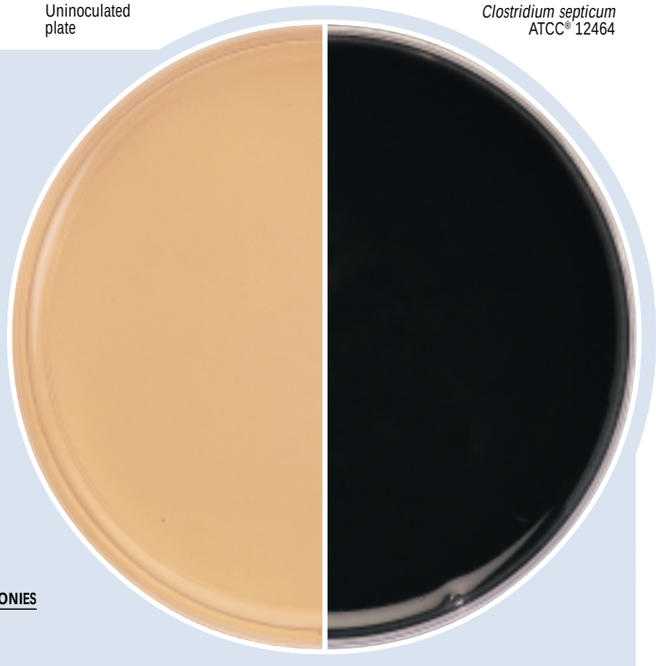
Differential Reinforced Clostridial Agar is also known as DRCA.

Summary and Explanation

Differential Reinforced Clostridial Medium was developed by Gibbs and Freame in 1965[1]. The medium could be used to enumerate clostridia in foods using the Most Probable Number (MPN) method.

Differential Reinforced Clostridial Agar (DRCA) is based on Differential Reinforced Clostridial Medium, but with the addition of agar.

The assay is performed using unheated and heat shocked tubes of DRCA containing replicate dilutions of the test sample. Blackening of the medium is presumptive evidence for the presence of sulfite-reducing clostridia. In this method, heat shocked tubes showing blackening are confirmatory for clostridia. Non-heat shocked tubes showing blackening



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.25% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, clear to slightly opalescent while hot; upon cooling, solution becomes light red.
Prepared Medium:	Light pink, very slightly to slightly opalescent.
Reaction of 4.25% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare Differential Reinforced Clostridial Agar per label directions. Inoculate and incubate at 35°C in an anaerobic environment for 72 hours.

ORGANISM	ATCC®	APPROXIMATE INOCULUM CFU	RECOVERY	BLACK COLONIES
Clostridium bifermentans	638	100	Good	+
Clostridium perfringens	12924	100	Good	+
Clostridium septicum	12464	100	Good	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

must be heat shocked to kill off vegetative cells and subcultured into DRCA to confirm the presence of sulfite-reducing clostridia.

Principles of the Procedure

Tryptone, Bacto Peptone, Beef Extract, Yeast Extract, Starch, and L-Cysteine provide nutrients and co-factors required for good growth of clostridia. Dextrose is included in the medium as an energy source. Partial selectivity of the medium is achieved through the addition of Sodium Acetate. Bacto Agar has been incorporated into this medium as a solidifying agent. Anaerobiosis in the medium is detected by the redox indicator Resazurin. The addition of Ferric Ammonium Citrate to the medium is used to detect sulfite reduction. Blackening of the medium is due to the formation of iron sulfide.

Formula

Differential Reinforced Clostridial Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Peptone	5 g
Bacto Beef Extract, Desiccated	8 g
Bacto Yeast Extract	1 g
L-Cysteine HCl	0.5 g
Starch	1 g
Dextrose	1 g
Sodium Acetate	5 g
Sodium Bisulfite	0.5 g
Ferric Ammonium Citrate	0.5 g
Resazurin	0.002 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Differential Reinforced Clostridial Agar

Material Required But Not Provided

Anaerobic Jar Complete
Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35°C)
Ringer's solution or 0.1% peptone water

Method of Preparation

1. Suspend 42.5 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 10 ml portions into tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect samples in sterile containers and transport immediately to the laboratory following recommended guidelines.[4,5]
2. Process each sample using procedures appropriate for that sample.[4,5]

Test Procedure

1. Prepare serial 10-fold dilutions of the sample in 1/4 strength Ringer's solution or 0.1% peptone water.
2. Depending on the amount of the initial sample, transfer 1 ml or 0.1 ml of the appropriate dilution, prepared in step 1, to the bottom of a molten (45-50°C) DRCA tube. Prepare a duplicate tube using the same procedure.
3. Tighten the caps on the tubes.
4. Heat one of the duplicate DRCA tubes prepared in step 2 to 80 ± 1°C for 10 minutes to kill vegetative cells.
5. Incubate both tubes, heat-shocked and non-heat-shocked, at 35 ± 1°C for 5 days; examine for sulfite reduction.

Results

The presence of clostridia is presumptively indicated by blackening in the medium. Heat-shocked tubes showing blackening should be considered confirmatory for the presence of sulfite-reducing clostridia.

Limitations of the Procedure

1. Non-heat-shocked cultures showing blackening must be heat shocked and subcultured to DRCA for confirmation.

References

1. **Gibbs, B. M., and B. Freame.** 1965. Methods for the recovery of clostridia from foods. J. Appl. Microbiol. **28:**95-143.
2. **Miller, N. J., O. W. Gerrett, and T. S. Prickett.** 1939. Anaerobic technique, a modified deep agar shake. Food Research **4:**447-51.
3. Mikrobiologische Untersuchungsverfahren gemäß Anlage 3 (zu § 4 Abs. 3) der Mineral-und Tafelwasserverordnung vom 1.8. 1984, Untersuchung auf sulfitreduzierende, sporenbildende Anaerobier.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Differential Reinforced Clostridial Agar	500 g	0641-17

Bacto® Dubos Albumin Broth · Bacto Dubos Broth Base Bacto Dubos Medium Albumin · Bacto Dubos Oleic Agar Base Bacto Dubos Oleic Albumin Complex

Intended Use

Bacto Dubos Albumin Broth is used for rapidly cultivating *Mycobacterium tuberculosis*.

Bacto Dubos Broth Base is used with Bacto Dubos Medium Albumin for rapidly cultivating pure cultures of *Mycobacterium tuberculosis*.

Bacto Dubos Oleic Agar Base is used with Bacto Dubos Oleic Albumin Complex and penicillin for isolating and determining the susceptibility of *Mycobacterium tuberculosis*.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] During the mid 1980s, the number of tuberculosis (TB) cases in the U.S. began increasing. Prior to this time, the number of cases in the U.S. had been decreasing, reaching a low in 1984.[2] Non-tuberculous mycobacteria infections have also increased since the mid 1980s.[3]

Dubos Broth is prepared according to the Dubos, Fenner and Pierce[4] modification of the medium originally described by Dubos and Davis[5] and Dubos and Middlebrook.[6]

Dubos and Middlebrook[6] described Dubos Oleic Medium Albumin as suitable for primary isolation and cultivation of the tubercle bacillus and for studying colony morphology. In comparative studies, Dubos Oleic Albumin Agar Medium was superior to other media studied for primary isolation.[7,8]

There are two types of solid culture media for the primary isolation of mycobacteria, those that have coagulated egg as a base and those that have agar. Lowenstein formulations are examples of media that contain egg; Middlebrook and Dubos formulations contain agar.

Agar based media are not liquified by contaminating proteolytic organisms but overgrowth may occur. These media are recommended for specimens from nonsterile sites.[9] The medium is clear so colonies of mycobacteria can be viewed through a stereo microscope even if contaminating organisms are present. Colonies can be observed in 10 to 12 days.

Drugs may be added to Dubos media in exact concentrations because the medium is solidified with agar rather than by inspissation. Also, there is less drug inactivation when egg ingredients are not present.

Mycobacteria grow more rapidly in broth media. Primary culture of all specimens in broth media is recommended.[10] Tween 80 in the medium acts as a surfactant, dispersing the bacilli, which increases growth.

User Quality Control

Identity Specifications

Dubos Albumin Broth

Appearance:	Almost colorless, clear to very slightly opalescent.
Reaction of Solution at 25°C:	pH 7.0 ± 0.2

Dubos Broth Base

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	0.65% solution, soluble in distilled or deionized water. Solution is very light to light amber, clear, may have a slight precipitate.
Reaction of 0.65% Solution at 25°C:	pH 6.6 ± 0.2

Dubos Medium Albumin

Appearance:	Very light amber, clear liquid.
Reaction of Solution at 25°C:	pH 6.6 ± 0.2

Dubos Oleic Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, slightly opalescent to opalescent with fine precipitate.
Reaction of 2% Solution at 25°C:	pH 6.6 ± 0.2

Dubos Oleic Albumin Complex

Appearance:	Light amber, clear liquid without precipitate.
Reaction of Solution at 25°C:	pH 6.8 ± 0.2

continued on following page

Principles of the Procedure

Casitone and Asparagine are sources of nitrogen. Disodium Phosphate and Monopotassium Phosphate are sources of phosphates and, along with Calcium Chloride, help maintain the pH of the medium. Magnesium Sulfate, Ferric Ammonium Sulfate, Zinc Sulfate and Copper Sulfate are sources of trace metals and sulfates. Bacto Agar is the solidifying agent.

Formula

Dubos Albumin Broth

Formula Per Liter		
Bacto Dubos Broth Base	6.5	g
Distilled or Deionized Water	900	ml
Bacto Dubos Medium Albumin	100	ml

Dubos Broth Base

Formula Per Liter

Bacto Casitone	0.5 g
Bacto Asparagine	2 g
Tween® 80	0.2 g
Monopotassium Phosphate	1 g
Disodium Phosphate (Anhyd.)	2.5 g
Ferric Ammonium Citrate	50 mg
Magnesium Sulfate	10 mg
Calcium Chloride	0.5 mg
Zinc Sulfate	0.1 mg
Copper Sulfate	0.1 mg

Dubos Medium Albumin

A 5% solution of albumin fraction V from bovine plasma and 7.5% dextrose in normal saline.

Dubos Oleic Agar Base

Formula Per Liter

Bacto Casitone	0.5 g
Bacto Asparagine	1 g
Monopotassium Phosphate	1 g
Disodium Phosphate (Anhyd.)	2.5 g
Ferric Ammonium Citrate	50 mg
Magnesium Sulfate	10 mg
Calcium Chloride	0.5 mg
Zinc Sulfate	0.1 mg
Copper Sulfate	0.1 mg
Bacto Agar	15 g

User Quality Control

Cultural Response

Dubos Albumin Broth

Prepare medium from Dubos Broth Base and Dubos Medium Albumin per label directions or use prepared Dubos Albumin Broth. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium fortuitum	6841	300-1,000	good
Mycobacterium intracellulare	13950	300-1,000	good
Mycobacterium kansasii	12478	300-1,000	good
Mycobacterium scrofulaceum	19981	300-1,000	good
Mycobacterium tuberculosis H37 Ra	25177	300-1,000	good

Dubos Oleic Agar

Prepare medium from Dubos Oleic Agar Base and Dubos Oleic Albumin Complex per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	1,000-2,000	partial inhibition
Mycobacterium fortuitum	6841	300-1,000	good
Mycobacterium intracellulare	13950	300-1,000	good
Mycobacterium kansasii	12478	300-1,000	good
Mycobacterium scrofulaceum	19981	300-1,000	good
Mycobacterium tuberculosis H37 Ra	25177	300-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Dubos Oleic Albumin Complex

A 0.05% solution of alkalinized oleic acid in a 5% solution of albumin fraction V in normal saline (0.85%).

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.
3. **Dubos Broth Base**

   IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   Dubos Oleic Agar Base

   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

Storage

Store **Dubos Broth Base** and **Dubos Oleic Agar Base** dehydrated below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store **Dubos Albumin Broth**, **Dubos Medium Albumin** and **Dubos Oleic Albumin Complex** at 2-8°C.

Store prepared media at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Dubos Albumin Broth
Dubos Broth Base
Dubos Medium Albumin
Dubos Oleic Agar Base
Dubos Oleic Albumin Complex

Materials Required but not Provided

Glycerol
Penicillin (for preparing Dubos Oleic Agar Base)
Glassware
Distilled or deionized water
Autoclave
Incubator (CO_2, 35°C)

Method of Preparation

Dubos Broth

1. Dissolve 1.3 grams **Dubos Broth Base** in 180 ml distilled or deionized water (or 170 ml water and 10 ml Glycerol).

2. Autoclave at 121°C for 15 minutes.
3. Cool below 50°C.
4. Aseptically add 20 ml **Dubos Medium Albumin** and mix thoroughly.
5. Dispense into tubes.

Dubos Oleic Agar

1. Suspend 4 grams **Dubos Oleic Agar Base** in 180 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50-55°C.
5. Aseptically add 20 ml **Dubos Oleic Albumin Complex** and 5,000 -10,000 units penicillin (25-50 units per ml medium).
6. Mix thoroughly.
7. Dispense into sterile tubes or plates.

Specimen Collection and Preparation[7]

1. Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each specimen as appropriate for that specimen.

Test Procedure

1. Inoculate the specimen onto/into the medium and incubate tubes for up to eight weeks.
2. Examine tubes for growth.

Results

Mycobacteria grow on the medium or in the broth.

Limitations of the Procedure

1. Negative culture results do not rule out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material, i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should remain loose for a free exchange of CO_2.

References

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2. **Klietmann, W.** 1995. Resistance and susceptibility testing for *Mycobacterium tuberculosis.* Clin. Microbiol. Newsletter **17**:65-69.
3. **Nolte, F. S., and B. Methcock**. 1995. *Mycobacterium,* p. 400-437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. Am. Rev. Tuberculosis, 1950, **61**:66.
5. J. Exp. Med., 1946, **83**:409.
6. Am. Rev. Tuberc., 1947, **56**:334.
7. A. Rev. Tuberculosis, 1950, **61**:563.
8. Am. J. Clin. Path., 1950, **20**:678.
9. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, suppl. 1. American Society for Microbiology, Washington, D.C.
10. **Tenover, F. C., J. T. Crawford, R. E. Huebner, L. J. Geiter, C. R. Horsburgh, Jr., and R. C. Good**. 1993. The resurgence of tuberculosis: is your laboratory ready? J. Clin. Microbiol. **31**:767-770.

Packaging

Dubos Albumin Broth	20	tubes	1022-39
Dubos Broth Base	500	g	0385-17
Dubos Medium Albumin	12 x 20	ml	0309-64
Dubos Oleic Agar Base	500	g	0373-17
Dubos Oleic Albumin Complex	12 x 20	ml	0375-64

Bacto® m E Agar
Bacto Esculin Iron Agar

Intended Use

Bacto m E Agar is used with nalidixic acid and triphenyl tetrazolium chloride in isolating and differentiating enterococci from water by membrane filtration and in an *in situ* esculin test on Bacto Esculin Iron Agar.

Bacto Esculin Iron Agar is used for enumerating enterococci from water by membrane filtration based on esculin hydrolysis.

Also Known As

Esculin Iron Agar is abbreviated as EIA.

Summary and Explanation

Enterococcus species are a subgroup of fecal streptococci that includes *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. avium*.[1] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10°C and 45°C.[1] The enterococci portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1]

Slanetz and Bartley[2] first reported quantitating enterococci by the membrane filter method in 1957. A wide range of levels of enterococci in water can be enumerated and detected because small or large volumes of water can be analyzed by the membrane filter technique.[3] In 1961, Kenner et al.[4] described the KF method for detecting and quantitating fecal streptococci. In 1966, Isenberg et al.[5] reported a plating procedure with differentiation based on esculin hydrolysis. Levin, Fischer and Cabelli[6] compared the KF method with Isenberg's

plating method, and found the latter method resulted in better recovery of fecal streptococci. They developed m E Agar as a primary isolation medium for enterococci, and Esculin Iron Agar as an *in situ* substrate test medium for identifying organisms capable of hydrolyzing esculin.[6]

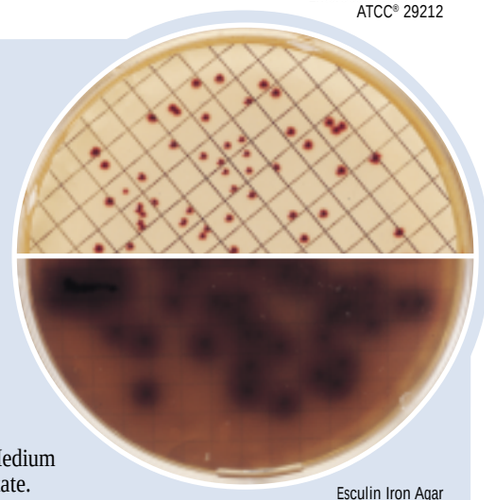
Two research projects by the Environmental Protection Agency (EPA) evaluated the relationships between swimming-associated illness and the ambient densities of indicator bacteria.[7,8] The studies demonstrated that enterococci have a better correlation with swimming-associated illness for both marine and fresh waters than fecal coliforms. *Escherichia coli* has a correlation in fresh water equal to enterococci but does not correlate as well in marine waters.[7,8] This suggests that enterococci may be better indicator organisms for some recreational waters.[7,8]

m E Agar and Esculin Iron Agar are prepared according to the formulas specified in Standard Methods.1 These media are used in the membrane filter technique for the isolation of fecal streptococcus and enterococcus groups.[1] This procedure can be used to test marine and fresh water sources.

m E Agar with the addition of 0.075% indoxyl B-D glucoside (m EI Agar) is recommended by the U.S. EPA as a one step procedure for the isolation and identification of enterococci in recreational water.[9] This method is used in the EPA Beaches Environmental Assessment Closure and Health (BEACH) Program. m EI Agar eliminates the necessity of transferring the incubated membrane to Esculin Iron Agar.

Principles of the Procedure

m E Agar is a highly selective and differential primary isolation medium that supports good growth of enterococci. Bacto Peptone and Yeast Extract provides carbon, nitrogen, minerals, vitamins and other growth factors for organism growth. Sodium Chloride maintains the osmotic balance of the medium. Nalidixic Acid and Sodium Azide act as selective agents to inhibit gram negative bacteria. Actidione® inhibits fungi. At the concentration in the formula, 2,3,5 triphenyl tetrazolium chloride (TTC) dyes enterococci colonies. TTC slightly inhibits growth of other microorganisms. In addition, the elevated incubation temperature of 41°C inhibits some indigenous microbial flora. Esculin is hydrolyzed by enterococci to form esculetin and dextrose. The esculetin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a black to reddish brown complex that appears in the medium surrounding the colonies. The production of black to reddish brown complex verifies the colonies as enteroccci and facilitates their enumeration. Bacto Agar is the solidifying agent in the medium.



m E Agar
Enterococcus faecalis
ATCC® 29212

Esculin Iron Agar
Enterococcus faecalis
ATCC® 29212

User Quality Control

Identity Specifications

m E Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	7.12% solution, soluble in distilled or deionized water upon boiling. Light to medium amber with bluish cast, very slightly opalescent.
Prepared Medium:	Light to medium amber with blue cast, slightly opalescent.
Reaction of 7.12% Solution at 25°C:	pH 7.1 ± 0.2

Esculin Iron Agar

Dehydrated Appearance:	Tan to dark tan, free-flowing, homogeneous.
Solution: amber with	1.65%, soluble in distilled or deionized water upon boiling. Medium blue cast, very slightly opalescent without significant precipitate.
Prepared Medium:	Medium amber with blue cast, slightly opalescent without precipitate.
Reaction of 1.65% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare m E Agar per label directions and pour into 9 x 50 mm plates. Dilute the test organisms and filter through membrane filters. Place the filters on m E Agar plates and incubate the plates in an upright position for 48 hours at 41 ± 0.5°C. Remove the filters and place over prepared Esculin Iron Agar plates. After 20 minutes incubation at 41 ± 0.5°C, count colonies giving positive esculin reaction (formation of black or reddish brown precipitate).

ORGANISM	ATCC®	INOCULUM CFU/10 ml	GROWTH ON m E AGAR	REACTION ON ESCULIN IRON AGAR
Enterococcus faecalis	29212*	20-60	good/pink to red colonies	black or red/brown ppt
Enterococcus faecalis	33186	20-60	good/pink to red colonies	black or red/brown ppt
Escherichia coli	25922*	20-60	marked to complete inhibition	inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

m E Agar

Formula Per Liter

Bacto Yeast Extract	30 g
Bacto Peptone	10 g
Sodium Chloride	15 g
Esculin	1 g
Actidione®	0.05 g
Sodium Azide	0.15 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Esculin Iron Agar

Formula Per Liter

Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **m E Agar**

   HARMFUL BY INHALATION AND IF SWALLOWED. (US) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

m E Agar
Esculin Iron Agar

Materials Required But Not Provided

Bacto TTC Solution 1%
Nalidixic acid
Indoxyl β-D glucoside (optional)
Sterile Petri dishes, 50 x 9 mm
Membrane filter equipment
Sterile pipettes
Sterile 47 mm, 0.45 µm, gridded membrane filters
Autoclave
Glassware
Dilution blanks
41°C incubator or waterbath
Fluorescent lamp
Magnifying lens

Method of Preparation

m E Agar

1. Suspend 7.12 grams in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45°C.
4. Add 0.024 grams of nalidixic acid and 1.5 ml TTC Solution 1% (0.015 grams triphenyl tetrazolium chloride).
5. Adjust to pH 7.1 if necessary.
6. Dispense 4-5 ml into 9 x 50 mm Petri dishes.

Note: Nalidixic acid is soluble in water with an alkaline pH.

Esculin Iron Agar

1. Suspend 1.65 grams in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense 4-5 ml into 9 x 50 mm Petri dishes.

Specimen Collection and Preparation

Collect water samples as described in Standard Methods for the Examination of Water and Wastewater.[1]

Test Procedure

1. Follow the membrane filter procedure described in Standard Methods for the Examination of Water and Wastewater.[1]
2. Choose a sample size so that 20-60 colonies will result.
3. Place the filter on an m E Agar plate and incubate for 48 hours at 41 ± 0.5°C.
4. After incubation, remove the filter from m E Agar and place it on Esculin Iron Agar plate. Retain at room temperature for approximately 20-30 minutes.
5. Incubate Esculin Iron Agar at 41 ± 0.5°C for 20 minutes.

Results

Pink to red enterococci develop a black or reddish-brown precipitate on the underside of the filter.[1] Count colonies using a fluorescent lamp and a magnifying lens.[1] Report results as estimated number or organisms per 100 ml of water.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. m E Agar and Esculin Iron Agar should be used in sequence.

3. Incubation at 41°C is recommended.
4. Approximately 10% false-positive esculin reactions may be expected. When used as m EI Agar, U.S. EPA reports a 6.0% false positive and 6.5% false negative rate with mE Agar.

References

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Packaging

m E Agar	100 g	0333-15
	500 g	0333-17
Esculin Iron Agar	100 g	0488-15

Bacto® EC Medium

Intended Use

Bacto EC Medium is used for differentiating and enumerating coliforms in water, wastewater, shellfish and foods.

Also Known As

EC Medium is also referred to as EC Broth. EC is an abbreviation for *Escherichia coli.*

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 No. 3. Growth of spore forming bacteria and fecal streptococci is inhibited by the bile salts, while growth of *E.coli* is enhanced by its presence. The medium can be

User Quality Control

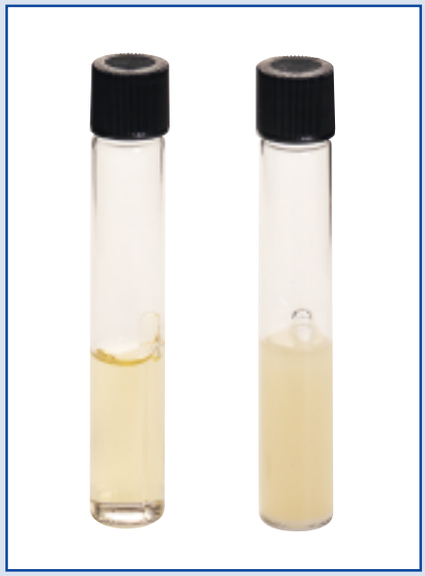
Identity Specifications

Dehydrated Appearance	Light beige, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water. Light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 3.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare EC Medium per label directions. Inoculate tubes with the test organisms, and incubate at 44.5 ± 0.2°C for 24 ± 2 hours. Read tubes for growth and gas production.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	GAS PRODUCTION
Enterococcus faecalis	19433	1,000	inhibited	–
Escherichia coli	25922*	1,000	good	+
Escherichia coli	8739	1,000	good	+



Uninoculated tube — *Escherichia coli* ATCC® 25922

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disk and should be used as directed in Bactrol Disk Technical Information.

used at 37°C for the detection of coliform organisms or at 45.5°C for the isolation of *E. coli*.

In a further evaluation of EC Medium and Lauryl Tryptose Broth, Perry and Hajna[2] reported the results obtained from eleven different laboratories examining a variety of waters, milk and shellfish. The results indicate that the media are highly specific for coliform bacteria. Fishbein and Surkiewicz[3] used the EC confirmation test for recovery of *E. coli* from frozen foods and nut meats. This study[3] showed that the test is optimal when conducted at 45.5°C, with incubation limited to 24 hours.

EC Medium is employed in elevated-temperature tests for distinguishing organisms of the total coliform group that also belong to the fecal coliform group.[4] 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 standard methods for food and water testing.[4,5,6]

Principles of the Procedure

Tryptose provides the nitrogen, vitamins and amino acids in EC Medium. Lactose is the carbon source. Bile Salts No. 3 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

EC Medium

Formula Per Liter

Bacto Tryptose	20 g
Bacto Lactose	5 g
Bacto Bile Salts No. 3	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

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

EC Medium

Materials Required But Not Provided

Glassware
Fermentation vials
Autoclave
Incubator or waterbath

Method of Preparation

1. Suspend 37 grams in 1 liter distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Obtain and process specimens according to the procedures established by laboratory policy or standard methods.[4,5,6]

Test Procedure

Follow the methods and procedures as stated in standard methods.[4,5,6]

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.[4]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents.[7]

References

1. **Hajna and Perry.** 1943. Am. J. Public Health **33**:550.
2. **Hajna and Perry.** 1944. Am. J. Public Health **34**:735.
3. **Fishbein and Surkiewicz.** 1964. Appl. Microbiol. **12**:127.
4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, 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 (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
7. **Ray, B.** 1986. Impact of bacterial injury and repair in food microbiology: Its past, present and future. J. Food Prot. **49**:651.

Packaging

EC Medium	100 g	0314-15
	500 g	0314-17
	10 kg	0314-08

Bacto® EC Medium with MUG

Intended Use

Bacto EC Medium with MUG is used for detecting *Escherichia coli* in water, food and milk.

Also Known As

EC is an abbreviation for *Escherichia coli*.

Summary and Explanation

EC Medium was developed by Hajna and Perry[1] to improve the methods for the detection of coliforms and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts No. 3. Growth of spores formers and fecal streptococci were inhibited by the bile salts, while growth of *E. coli* is enhanced. EC Medium with MUG is the same formula as EC Medium with the addition of 4-methylumbelliferyl-ß-D-glucuronide.

Feng and Hartman[2] developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-ß-D-glucuronide (MUG) into Lauryl Tryptose Broth at a final concentration of 100 µg/ml. Robison[3] compared the fluorogenic assay with present methodology and found that total agreement between the two methods was 94.8%. Moburg[4] determined the amount of MUG could be reduced to a final concentration of 50 µg/ml without adversely affecting results. Koburger and Miller[5] recommended the incorporation of MUG into EC Broth for use in testing shellfish.

EC Medium with MUG is prepared according to the formula specified by US EPA[6] and standard methods for water and food testing.[7,8]

Principles of the Procedure

Tryptose provides the nitrogen, vitamins and amino acids in EC Medium with MUG. Lactose is the carbon source in this medium. Bile Salts No. 3 is the selective agent against gram-positive bacteria, particularly bacilli and fecal streptococci. Dipotassium Phosphate and Monopotassium Phosphate are buffering agents. Sodium Chloride maintains the osmotic balance of the medium.

E. coli produces the enzyme glucuronidase that hydrolyzes MUG to yield a fluorogenic product that is detectable under long-wave (366 nm) UV light. The addition of MUG to EC Medium provides another criterion, in addition to growth response and gas production, to determine the presence of *E. coli* in food and environmental samples.

Formula

EC Medium with MUG

Formula Per Liter

Bacto Tryptose	20 g
Bacto Lactose	5 g
Bacto Bile Salts No. 3	1.5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g
MUG (4-methylumbelliferyl-ß-D-glucuronide)	0.05 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.71% solution, soluble in distilled or deionized water; light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 3.71% Solution at 25°C:	pH 6.9 ± 0.2

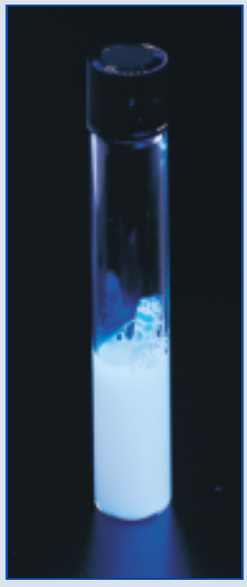
Cultural Response

Prepare EC Medium with MUG per label directions. Inoculate tubes in duplicate. Incubate the first set at 35 ± 2°C for 24 hours and the second set at 44.5 ± 0.2°C. Read fluorescence under a long-wave UV light.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH AT 35°C/GAS	GROWTH AT 44.5°C/GAS	FLUORESCENCE
Enterobacter aerogenes	13048*	100-1,000	good/+	inhibited/–	–
Escherichia coli	25922*	100-1,000	good/+	good/+	+
Enterococcus faecalis	19433*	100-1,000	inhibited/–	inhibited/–	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Escherichia coli
ATCC® 25922

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

EC Medium with MUG

Materials Required But Not Provided

Test tubes
Fermentation vials
Sterile pipettes
Incubator 35°C, 44.5°C
Long-wave UV lamp
Autoclave

Method of Preparation

1. Suspend 37.1 grams in 1 liter distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Dispense into test tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.
5. Before opening the autoclave, allow the temperature to drop below 75°C to avoid entrapping air bubbles in the fermentation vials.

Specimen Collection and Preparation

Collect food, water or other environmental samples in accordance with recommended procedures.[6,7,8]

Test Procedure

Follow the methods and procedures as stated in appropriate references.[6,7,8]

Results

Following incubation, observe tubes for growth, production of gas and fluorescence. Positive gas production is demonstrated by displacement of the medium from the fermentation vial. Positive MUG reactions exhibit a bluish fluorescence under long-wave (approximately 366 nm) UV light. Typical strains of *E. coli* are positive for both gas production and fluorescence. Non-*E. coli* coliforms that grow may exhibit fluorescence but will not produce gas.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Strains of *E. coli* that fail to grow in EC Medium with MUG, fail to produce gas, or fail to produce glucuronidase may infrequently be encountered.
3. Strains of *Salmonella*, *Shigella* and *Yersinia* that produce glucuronidase may be encountered. These strains must be distinguished from *E. coli* on the basis of other parameters, i.e., gas production, growth at 44.5°C.
4. The presence of endogenous glucuronidase in shellfish samples may cause false positive fluorescent reactions at the presumptive stage. To prevent this problem, the use of EC Medium with MUG in the confirmatory stage has been recommended.[5]

References

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. **Robison, B. J.** 1984. Evaluation of a fluorogenic assay for detection of *Escherichia coli* in foods. App. Environ. Microbiol. **48**:285-288.
4. **Moberg, L. J.** 1985. Fluorogenic assay for rapid detection of *Escherichia coli* in food. Appl. Environ. Microbiol. **50**:1383-1387.
5. **Koburger, J. A., and M. L. Miller.** 1985. Evaluation of a fluorogenic MPN procedure for determining *Escherichia coli* in oysters. J. Food Prot. **48**:244-245.
6. **Federal Register.** 1991. National primary drinking water regulation; analytical techniques; coliform bacteria. Fed. Regist. **56**:636-643.
7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
8. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

EC Medium with MUG	100 g	0022-15
	500 g	0022-17

Bacto® EE Broth Mossel

Intended Use

Bacto EE Broth Mossel is used for selectively enriching and detecting *Enterobacteriaceae*, particularly from foods.

Summary and Explanation

EE Broth Mossel is prepared according to the formula of Mossel, Visser and Cornelissen.[1] The formula contains dextrose to facilitate growth of most *Enterobacteriaceae*, thus insuring the detection of *Salmonella* and other lactose- negative organisms. EE Broth Mossel should be used as an enrichment broth, followed by a selective medium, e.g., Violet Red Bile Agar.

The enumeration of *Enterobacteriaceae* is of great concern in monitoring the sanitary condition of food. *Enterobacteriaceae* can be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers.[2] Recovery relies on proper resuscitation of damaged cells.

Principles of the Procedure

Tryptose provides nitrogen, vitamins and amino acids. Dextrose is a carbon source. Disodium Phosphate and Monopotassium

Phosphate are buffering agents. Brilliant Green and Oxgall are selective agents.

Formula

EE Broth Mossel

Formula Per Liter	
Bacto Tryptose	10 g
Bacto Dextrose	5 g
Disodium Phosphate	8 g
Monopotassium Phosphate	2 g
Brilliant Green	0.0135 g
Bacto Oxgall	20 g

Final pH 7.2± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

EE Broth Mossel

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath

Method of Preparation

1. Dissolve 45 grams in 1 liter distilled or deionized water.
2. Dispense 120 ml amounts into 250 ml flasks.
3. Heat at 100°C (waterbath or flowing steam) for 30 minutes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

1. Inoculate flasks of EE Broth Mossel with approximately 10 grams of homogenized food or other material to be tested.
2. Shake the inoculated medium thoroughly for a few seconds to mix well.
3. Incubate for a total of 20-24 hours at 35-37°C. Shake the flasks after the first 3 hours of incubation.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light green, homogeneous, free-flowing.
Solution:	4.5% solution, soluble in distilled or deionized water; emerald green and clear.
Prepared Medium:	Emerald green, clear.
Reaction of 4.5% Solution at 25°C	pH 7.2 ± 0.2

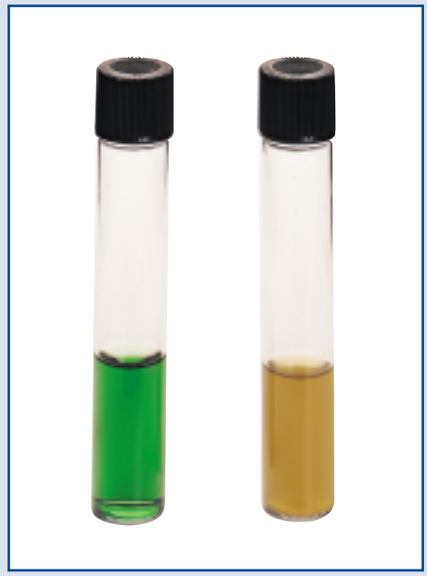
Cultural Response

Prepare EE Broth Mossel per label directions. Inoculate the medium and incubate at 35 ± 2°C for 18-24 hours and up to 48 hours if necessary.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Escherichia coli	25922*	100-1,000	good	+ (yellow)
Staphylococcus aureus	25923*	100-1,000	inhibited	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube

Escherichia coli ATCC® 25922

4. Prepare plates such as Violet Red Bile Agar for streaking. To ensure recovery of dextrose fermenters, add 1% dextrose before boiling.
5. Streak a loopful of the enrichment culture onto the prepared plates.
6. Incubate the plates for 18-24 hours at 35-37°C. Examine for the presence of coliforms which appear pink to purplish-red on Violet Red Bile Agar. The color of coliform colonies may vary if a different medium is used.

For a complete discussion on *Enterobacteriaceae* in food testing, refer to procedures in Standard Methods.[3,4]

Results

Acid production causes the color of EE Broth Mossel to become yellow. A negative reaction results in no color change and the medium remains green.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Mossel, D. A. A., M. Vissar, and A. M. R. Cornellisen.** 1963. The examination of foods for *Enterobacteriaceae* using a test of the type generally adopted for the detection of salmonellae. J. Appl. Bacteriol. **26**:444-452.
2. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of *Salmonella* in foods. J. Food Prot. **44**:385-386.
3. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

EE Broth Mossel	500 g	0566-17
	10 kg	0566-08

Bacto® EMB Agar

Intended Use

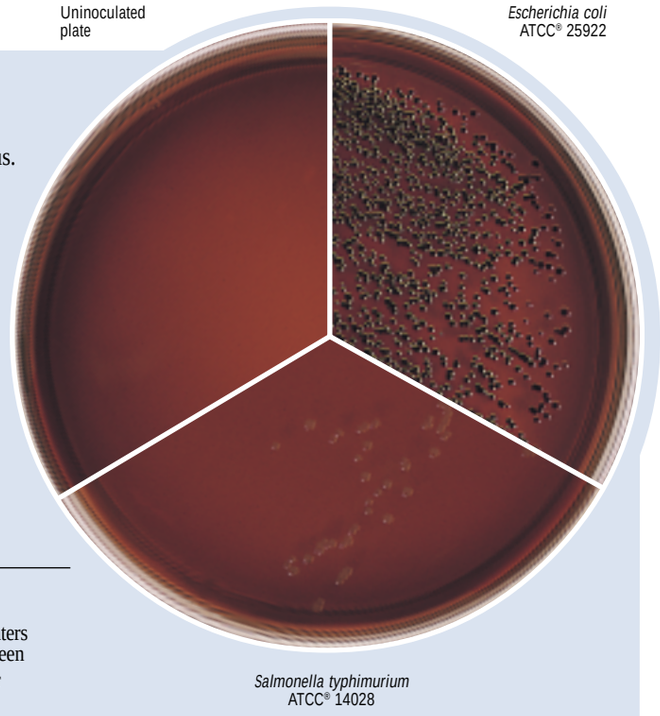
Bacto EMB Agar is used for isolating and differentiating gram-negative enteric bacilli.

Also Known As

EMB Agar is also known as Eosin Methylene Blue Agar

Summary and Explanation

The original Eosin Methylene Blue Agar was the formulation of Holt-Harris and Teague.[1] The use of eosin and methylene blue as indicators gave sharp and distinct differentiation between colonies of lactose fermenting and nonfermenting organisms. Sucrose was included in the medium to detect members of the coliform group that fermented sucrose more readily than lactose. Lactose-positive colonies were black



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish purple, free flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling. Solution is green with orange cast, opalescent with a uniform flocculent precipitate.
Prepared Plates:	Purple with a greenish-orange cast, opalescent, may have a fine precipitate.
Reaction of 3.6% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1,000	partial inhibition	colorless
Escherichia coli	25922*	100-1,000	good	blue-black w/dark centers and green metallic sheen
Salmonella typhimurium	14028*	100-1,000	good	colorless to amber

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

or possessed dark centers with transparent, colorless peripheries. Lactose- or sucrose-negative colonies were colorless. The Eosin Methylene Blue Agar of Holt-Harris and Teague had definite advantages over the Fuchsin Sulfite Agar of Endo. The EMB Agar formulation was more sensitive, more accurate, more stable, and gave an earlier differentiation between the lactose fermenters and lactose and sucrose nonfermenters.

Two years after Holt-Harris and Teague had introduced their new medium, Levine[2] described an Eosin Methylene Blue Agar for differentiating fecal and nonfecal coliforms. Levine's medium differentiated salmonellae and other lactose nonfermenters from the coliform organisms.

EMB Agar is a combination of the Levine and the Holt-Harris and Teague formulae. EMB Agar is selective due to the presence of inhibitors and differential based on the ability of some organisms to ferment carbohydrates with the absorption of eosin and methylene blue.

EMB Agar is recommended for use in examining clinical specimens for enteric pathogens.[3,4,5] The medium enables the isolation and differentiation of gram-negative enteric bacilli.

Principles of the Procedure

Peptone is a source of nitrogen and other nutrients in the formulation. Eosin and methylene blue are dyes which combine to form a precipitate at an acid pH. The dyes act both as pH indicators and inhibitors. Gram-positive bacteria are partially inhibited on the medium. Lactose and Sucrose are fermentable carbohydrates. Phosphate acts as a buffer. Bacto Agar is a solidifying agent.

Formula

EMB Agar

Formula Per Liter

Bacto Peptone	10 g
Bacto Lactose	5 g
Bacto Sucrose	5 g
Dipotassium Phosphate	2 g
Bacto Agar	13.5 g
Eosin Y	0.4 g
Methylene Blue	0.065 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared plates at 2-8°C.

Expiration Date

Expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

EMB Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50)°C
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Cool to 45-50°C in a waterbath.
5. Dispense into sterile Petri dishes. Evenly disperse the precipitate when dispensing.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[3,4,5]
2. For specific information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[3,4,5]

Test Procedure

For isolation of enteric pathogens from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the EMB Agar plate and streak for isolation. This will permit development of discrete colonies. Incubate plates at 35°C. Examine plates at 24 hours and again at 48 hours for colonies with characteristic morphologies associated with potential pathogens.

Results

Salmonella and *Shigella* colonies are translucent and amber colored or colorless. Coliforms that use lactose and/or sucrose produce blue-black colonies with dark centers and greenish metallic sheen. Other coliforms such as *Enterobacter* form mucoid, pink colonies. Strains of *Enterococcus faecalis* are partially inhibited on this medium and appear as colorless colonies.

Limitations of the Procedure

1. EMB Agar is only moderately inhibitory. Some staphylococci, streptococci and yeast may grow. They will appear as small, pinpoint colonies. Gram-negative nonfermenting bacilli may grow and appear as non-lactose fermenters. Biochemical tests are necessary for further identification to genus or species.[6]
2. Some strains of *Salmonella* and *Shigella* may not grow on EMB Agar.[6] It is recommended that a nonselective, differential medium (MacConkey Agar or Hektoen Enteric Agar) and a selective medium (Bismuth Sulfite Agar, SS Agar or Desoxycholate Citrate Agar) be run in parallel with EMB Agar.
3. Sterilization reduces the methylene blue, leaving the medium orange in color. The normal purple color of the medium may be restored by gentle mixing. If the reduced medium is not shaken to oxidize the methylene blue, a dark zone beginning at the top and extending downward through the medium will gradually appear. The sterilized medium normally contains a flocculent precipitate which should not be removed. By cooling to 50°C and gently

mixing the medium before pouring it into plates, the flocculation will be finely dispersed.

4. Greenish metallic sheen is **not** always present. The presence of the greenish metallic sheen is **not** diagnostic for *E. coli*.[6]
5. Store and incubate EMB Agar plates in the dark. Visible light can alter the ability of the medium to support microbial growth, especially of *Proteus* spp.[7]

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:**596-600.
2. **Levine, M. M.** 1918. Differentiation of *E. coli* and *B. aerogenes* on a simplified Eosin-Methylene Blue Agar. J. Infect. Dis. **23:**43.
3. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*, p. 450-456. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Pezzlo, M. (ed.).** 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.
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. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
7. **Girolami, R. L., and J. M. Stamm.** 1976. Inhibitory effect of light on growth-supporting properties of Eosin Methylene Blue Agar. Appl. Environ. Microbiol. **31:**141.

Packaging

EMB Agar	100 g	0076-15
	500 g	0076-17
	2 kg	0076-07
	10 kg	0076-08

Bacto® EVA Broth

Intended Use

Bacto EVA Broth is used for detecting and confirming enterococci in water and other specimens as an indication of fecal contamination.

Also Known As

EVA Broth is also known as Ethyl Violet Azide Broth.

Summary and Explanation

The presence of enterococci in water and other specimens indicates fecal contamination. Mallmann and Seligmann[1] compared various enrichment media for detecting fecal streptococci and found that Azide Dextrose Broth presumptively identified the streptococci. However, because gram-positive bacteria other than enterococci grow in the medium, confirmation is necessary. Litsky et al.[2] studied various dyes and selective agents and formulated a medium using ethyl violet and sodium azide as selective agents. The medium known as Ethyl Violet Azide (EVA) Broth is specific for enterococci. In conjunction with Azide Dextrose Broth, EVA Broth is used to confirm the presence of enterococci.

Principles of the Procedure

EVA Broth contains Tryptose as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate. Sodium Azide and

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.58% solution, soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.
Reaction of 3.58% Solution at 25°C:	pH 7.0 ± 0.2

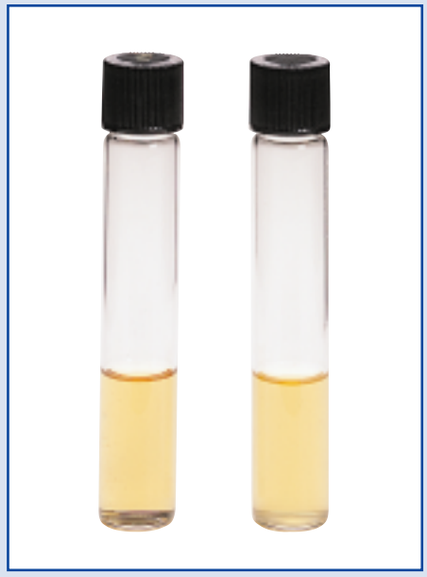
Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	19433*	100-1,000	good
Enterococcus faecalis	29212*	100-1,000	good
Escherichia coli	25922*	1,000-2,000	inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube — *Enterococcus faecalis* ATCC® 29212

Ethyl Violet inhibit gram-positive bacilli and gram-positive cocci other than enterococci. Monopotassium and Dipotassium Phosphates buffer the medium. Sodium Chloride provides osmotic balance.

Formula

EVA Broth

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	5 g
Dipotassium Phosphate	2.7 g
Monopotassium Phosphate	2.7 g
Sodium Chloride	5 g
Sodium Azide	0.4 g
Ethyl Violet	0.00083 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Nerves, Lungs, Cardiovascular.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

EVA Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 35.8 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Growth of enterococci.

References

1. **Mallmann and Seligmann**. 1950. Am. J. Pub. Health **40**:286.
2. **Litsky, Mallmann, and Fifield**. 1953. Am. J. Pub. Health **43**:873.

Packaging

EVA Broth	500 g	0606-17

Bacto® Egg Meat Medium

Intended Use

Bacto Egg Meat Medium is recommended for cultivating *Clostridium* cultures used in detecting the sporicidal activity of disinfectants.

Summary and Explanation

Egg Meat Medium is a dehydrated medium containing particles of meat, egg white and calcium carbonate.

The use of a combination meat and egg white culture medium was reported by Rettger[1] in his studies on *Escherichia coli* and *Enterobacter*

User Quality Control

Identity Specifications

Dehydrated Appearance:	Brown, free-flowing, homogeneous pellets.
Solution:	15% solution, insoluble in distilled or deionized water. Solution is a light to medium amber, clear to very slightly opalescent supernatant over insoluble pellets.
Reaction of 15% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Egg Meat Medium per label directions. Inoculate tubes and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis	19659*	100-1,000	good
Clostridium sporogenes	3584*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

aerogenes. Later, he described the use of this medium in studies of intestinal putrefaction.[2] In 1923, Reddish and Rettger[3] used the medium in their detailed study of *Clostridium putrificum* and, the following year, in a study of other spore-forming anaerobes.[4]

AOAC[5] recommends Egg Meat Medium to propagate and maintain cultures of *Clostridium* for use in testing sporicidal activity of liquid and gaseous chemicals.

Principles of the Procedure

Beef muscle provides carbon, nitrogen, inorganic salts, vitamins, and a variety of other nutrients to support bacterial growth. Egg whites are a source of protein. Calcium carbonate helps neutralize acids and displaces oxygen in the media.

Formula

Egg Meat Medium

Formula per liter

Beef Muscle, from	454 g
Egg White, from	6 eggs
Calcium Carbonate	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Egg Meat Medium

Materials Required but not Provided

Glassware
Garden soil extract (AOAC, 1995, 6.3.05, A.[a.][1.])[5]
Autoclave

Method of Preparation

1. Suspend 1.5 grams in 15 ml garden soil extract.[5] Let stand at least 15 minutes to form a thoroughly wetted, even suspension.
2. Autoclave at 121°C for 15 minutes. Allow to cool.
3. Avoid a rapid release of pressure after sterilization to prevent expelling the medium from the test tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Refer to AOAC[5] for detailed procedures to determine presence or absence of sporicidal activity of disinfectants against specified spore-forming bacteria.

Results

Refer to appropriate references and procedures for results.

References

1. **Rettger, L. F.** 1903. An experimental study of the chemical products of Bacillus coli communis and Bacillus lactis aerogenes. Am. J. Physiol. **8:**284.
2. **Rettger, L.** 1906. Studies on putrefaction. J. Biol. Chem. **2:**71.
3. **Reddish, G. F., and L. F. Rettger.** 1923. Clostridium putrificum. II. Morphological, cultural and biochemical study. J. Bact. **8:**375.
4. **Reddish, G. F., and L. F. Rettger.** 1924. A morphological, cultural and biochemical study of representative spore-forming anaerobic bacteria. J. Bact. **9:**13.
5. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Egg Meat Medium	500 g	0042-17

Bacto® Elliker Broth

Intended Use

Bacto Elliker Broth is used for cultivating streptococci and lactobacilli, particularly in dairy procedures.

Also Known As

Elliker Broth is also called Lactobacilli Broth.

Summary and Explanation

Testing for lactic acid bacteria in dairy products may be useful for various reasons.[3] These include determining the cause of acid defects in dairy products, evaluating lactic starter cultures, and controlling the quality of cured cheese, cultured milks, and uncultured products.[3] Lactic acid bacteria found in dairy products are primarily *Streptococcus*, *Lactococcus*, *Leuconostoc* and *Lactobacillus*.[3]

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.

A modification of Elliker Broth, Lactic (Elliker) Agar is recommended for general purpose enumeration of lactic acid bacteria.[3]

Principles of the Procedure

Tryptone and Gelatin provide the nitrogen and amino acids in Elliker Broth. Yeast Extract is the vitamin source in this formula. Dextrose, Lactose and Saccharose are the fermentable carbohydrates. Sodium

Chloride maintains the osmotic balance of the medium, and ascorbic acid is added to create a proper environment for organism growth. Sodium Acetate is a selective agent against gram negative bacteria.

Formula

Elliker Broth

Formula Per Liter

Bacto Tryptone	20 g
Bacto Yeast Extract	5 g
Bacto Gelatin	2.5 g
Bacto Dextrose	5 g
Bacto Lactose	5 g
Bacto Saccharose	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

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.85% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, clear without significant precipitate.
Prepared Medium:	Light to medium amber, clear without significant precipitate.
Reaction of 4.85% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Elliker Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours except *Streptococcus cremoris* which is incubated at 30 ± 2°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus casei	7469	100-1,000	good
Lactobacillus delbrueckii subsp. *lactis*	8000	100-1,000	good
Streptococcus cremoris	9596	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Procedure

Materials Provided

Elliker Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile tubes

Method of Preparation

1. Suspend 48.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of streptococci and lactobacilli, refer to standard methods in food testing.[3,4,5]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

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 (ed.).** 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.

Packaging

Elliker Broth	500 g	0974-17

Bacto® Emerson YpSs Agar

Intended Use

Bacto Emerson YpSs Agar is used for cultivating *Allomyces* and other fungi.

Summary and Explanation

Emerson YpSs Agar is prepared according to the formula given by Emerson.[1] Emerson and Wilson[2] used the medium at half strength for streaking zygotes or zoospores to obtain single germlings.

Fungi are extremely successful organisms, as evidenced by their ubiquity in nature.[3] Of the estimated 250,000 species, fewer than 150 are known primary human pathogens.[3] Opportunistic fungal pathogens are increasing at an impressive rate relating directly to the expanding size of the immunocompromised to patient population.[3]

Principles of the Procedure

Yeast Extract provides a source of trace elements, vitamins and amino acids. Soluble Starch provides starch for hydrolysis, detoxification of metabolic byproducts and as a carbon source. Dipotassium Phosphate is a buffer. Magnesium Sulfate is a source of divalent cations and sulfate. Bacto Agar is the solidifying agent.

Formula

Emerson YpSs Agar

Formula Per Liter

Bacto Yeast Extract	4 g
Bacto Soluble Starch	15 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Bacto Agar	20 g

Final pH 7.0 + 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.05% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Reaction of 4.05% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Emerson YpSs Agar per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Allomyces macrogynus	38327	100-300	good
Allomyces reticulatus	42465	100-300	good
Aspergillus niger	16404	100-300	good
Saccharomyces cerevisiae	9763	100-300	good

The cultures listed are the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Emerson YpSs Agar

Materials Required But Not Provided

Glassware
Autoclave
Sterile Petri dishes
Waterbath (optional)

Method of Preparation

1. Suspend 40.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and cultivation of fungi.

Results

Refer to appropriate references and procedures for results.

References

1. **Lloydia.** 1941. **4**:77.
2. **Mycologia.** 1954. **46**:393.
3. **Dixon, D. M., and R. A. Fromtling.** 1995. Morphology, taxonomy, and classification of the fungi, p. 699-708. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Emerson YpSs Agar	500 g	0739-17

Bacto® Endo Agar

Intended Use

Bacto Endo Agar is used for confirming the presence of coliform organisms.

Summary and Explanation

Endo[1] developed a medium using a fuchsin sulfite indicator to differentiate lactose fermenting and lactose non-fermenting organisms. Coliform organisms that ferment lactose produce red colonies and color the surrounding medium. Typical reactions of this medium are not caused by acid production but by the intermediate product acetaldehyde, which reacts with sodium sulfite.[2-3]

Endo Agar was formerly a standard methods medium for the microbiological examination of water[4] and dairy products.[5]

Principles of the Procedure

Lactose-fermenting bacteria produce acetaldehyde. The aldehyde is fixed by the sodium sulfite and in the presence of fuchsin forms red colonies. A sheen is produced by rapid lactose fermenting organisms. Lactose non-fermenting bacteria form clear, colorless colonies.

Endo Agar contains Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Lactose is the carbohydrate source. Basic Fuchsin in the presence of Sodium Sulfite produces the red colonies. Bacto Agar is the solidifying agent.

Formula

Endo Agar

Formula Per Liter

Bacto Peptone	10 g
Bacto Lactose	10 g
Dipotassium Phosphate	3.5 g
Bacto Agar	15 g
Bacto Basic Fuchsin	0.5 g
Sodium Sulfite	2.5 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Liver, Thyroid.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium purple, free-flowing, homogeneous.
Solution:	4.15% solution, soluble in distilled or deionized water on boiling. Solution is pink, slightly opalescent, may have a slight precipitate.
Prepared Medium:	Pink, slightly opalescent, may have a slight precipitate.
Reaction of 4.15% Solution at 25°C:	pH 7.5 ± 0.2

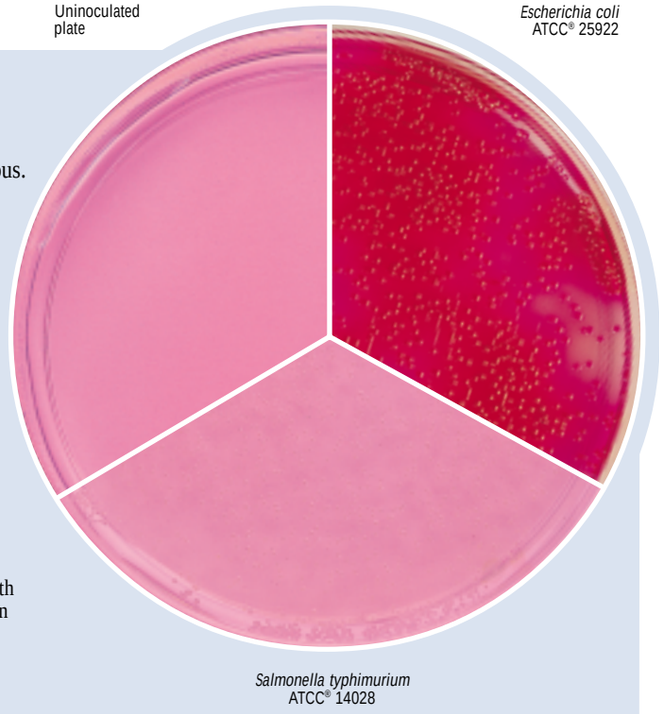
Cultural Response

Prepare Endo Agar per label directions. Inoculate plates and incubate at 35 ± 2°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	100-1,000	good	pink to red with metallic sheen
Salmonella typhimurium	14028*	100-1,000	good	colorless to light pink
Staphylococcus aureus	25923	1,000-2,000	marked to complete inhibition	

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Endo Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 41.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Evenly disperse the precipitate when dispensing.
5. Use immediately.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

See appropriate references for specific procedures.

Results

Rapid lactose fermenting organisms will produce red colonies that have a metallic sheen. Slow lactose fermenting organisms will produce red colonies. Lactose non-fermenting organisms will produce colorless colonies.

Limitations of the Procedure

If the medium is to be used the same day it is rehydrated, it does not need to be autoclaved. Boil to dissolve completely before dispensing into plates.

References

1. **Endo, S.** 1904. Uber ein Verfahren zum Nachweis der Typhusbacillen. Centr. Bakt., Abt 1, Orig. **35**:109-110.
2. **Margolena, L. A., and P. A. Hansen.** 1933. The nature of the reaction of the colon organism on Endo's medium. Stain Tech. **8**:131-139.
3. **Neuberg, C., and F. F. Nord.** 1919. Anwendungen der abfangmethode auf die bakteriengarungen. Biochem. Zeit. **96**:133-174.
4. **American Public Health Association**. 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
5. **American Public Health Association**. 1972. Standard methods for the examination of dairy products, 13th ed. American Public Health Association, Washington, D.C.

Packaging

Endo Agar	500 g	0006-17

Bacto® m Endo Agar LES

Intended Use

Bacto m Endo Agar LES is used for enumerating coliforms in water by membrane filtration.

Also Known As

LES (Lawrence Experimental Station) Endo Agar

Summary and Explanation

McCarthy, Delaney and Grasso[1] formulated Endo Agar LES (Lawrence Experimental Station) for testing water for coliform bacteria by a two-step membrane filter procedure using Lauryl Tryptose Broth as a preliminary enrichment. They recovered higher numbers of coliforms by this method compared with the one step technique using m Endo Broth.

The American Public Health Association specifies using m Endo Agar LES in the standard total coliform membrane filtration procedure for testing drinking water[2] and bottled water.[3] It is also specified for use in the completed phase of the standard total coliform fermentation technique.[2] The coliform bacteria are bacteria that produce a red colony with a metallic (golden) sheen within 24 hours incubation at 35°C on an Endo-type medium.

The U. S. Environmental Protection Agency specifies using m Endo Agar LES in the total coliform methods for testing water using single-step, two-step and delayed incubation membrane filtration methods.[4,5]

Principles of the Procedure

m Endo Agar LES contains tryptose, casitone and thiopeptone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins, which stimulate bacterial growth. Lactose is the carbohydrate. Phosphates are buffering agents. Sodium Chloride maintains the osmotic balance of the medium. Sodium Desoxycholate and Sodium Lauryl Sulfate are added as inhibitors. Basic Fuchsin is a pH indicator. Sodium Sulfite is added to decolorize the Basic Fuchsin solution. Bacto Agar is the solidifying agent.

Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulfite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism produces aldehydes with the rapid fermentation of lactose. If the inoculum is too heavy, the sheen will be suppressed. Lactose non-fermenting bacteria form clear, colorless colonies.

Formula

m Endo Agar LES

Formula Per Liter

Bacto Yeast Extract	1.2 g
Bacto Casitone	3.7 g
Thiopeptone	3.7 g
Bacto Tryptose	7.5 g
Bacto Lactose	9.4 g
Potassium Phosphate Dibasic	3.3 g
Potassium Phosphate Monobasic	1 g
Sodium Chloride	3.7 g
Sodium Desoxycholate	0.1 g
Sodium Lauryl Sulfate	0.05 g
Sodium Sulfite	1.6 g
Bacto Basic Fuchsin	0.8 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Liver, Thyroid.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Purple, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in distilled or deionized water containing 2% ethanol on boiling. Solution is pinkish-red, slightly opalescent to opalescent with precipitate.
Prepared Medium:	Rose colored, slightly opalescent, with precipitate.
Reaction of 5.1% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare m Endo Agar LES per label directions. Use the membrane filter technique to inoculate filters and preincubate on pads saturated with Lauryl Tryptose Broth (0241) at 35 ± 2°C for 1 1/2-2 hours. Transfer filters to plates of m Endo Agar LES and incubate at 35 ± 2°C for 22 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	20-80	good	red with sheen
Salmonella typhimurium	14028	20-80	good	pink
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

m Endo Agar LES
Lauryl Tryptose Broth
Materials Required but not Provided
95% Ethanol (Not denatured)
Glassware
Distilled or deionized water
Membrane filter apparatus
Membrane filter absorbent pads
Petri dishes, 60 mm
Incubator (35°C)

Method of Preparation

m Endo Agar LES

1. Suspend 51 grams in 1 liter distilled or deionized water containing 20 ml ethanol (95% not denatured).
2. Boil to dissolve completely. **Do Not Autoclave**.
3. Dispense in 5-7 ml quantities into 60 mm sterile petri dishes.

Lauryl Tryptose Broth

1. Suspend 35.6 g in 1 liter of distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Collect samples and process according to recommended guidelines for enumerating coliforms in water.[2,4,5]

Test Procedure

1. Place a membrane filter absorbent pad inside the cover.
2. Add 1.8-2.0 ml Lauryl Tryptose Broth to each pad.
3. Run the water sample through a membrane filter.
4. Place the filter, top side up, onto the pad containing Lauryl Tryptose Broth. Use a rolling motion to avoid entrapping air bubbles.
5. Incubate at 35 ± 2°C for 1½-2½ hours. Transfer the membrane from the pad to the surface of the m Endo Agar LES medium in the petri dish bottom, keeping the side on which the bacteria have been collected facing upward.

6. Leave the filter pad in the lid and incubate the plates in the inverted position at 35 ± 2°C for 22 ± 2 hours.
7. Observe and count all colonies that are red and have a metallic sheen.

Results

All colonies that are red and have the characteristic metallic sheen are considered coliforms. The sheen may cover the entire colony, may only be in the center or may appear only around the edges.

Limitations of the Procedure

1. Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies (dark red or nucleated colonies without sheen). It is advisable to verify both colony types.[2]

References

1. **McCarthy, J. A., J. E. Delaney, and R. J. Grasso**. 1961. Measuring coliforms in water. Water Sewage Works. **108**:238.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg** (ed.), 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 (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Bordner, R., and J. Winter (ed.)**, 1978. Microbiological methods for monitoring the environment, water and wastes. 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.

Packaging

m Endo Agar LES	100 g	0736-15
	500 g	0736-17
Lauryl Tryptose Broth	100 g	0241-15
	500 g	0241-17
	2 kg	0241-07
	10 kg	0241-08

Bacto® m Endo Broth MF®

Intended Use

Bacto m Endo Broth MF® is used for enumerating coliform organisms in water by membrane filtration.

Also Known As

m Endo Medium. MF is a registered trademark of Millipore Filter.

Summary and Explanation

Bacto m Endo Broth MF is prepared according to the formulation of the Millipore Filter Corporation.[1] for selectively isolating coliform bacteria from water and other specimens using the membrane filtration technique. The medium is a combination of the former m HD Endo Medium and Lauryl Tryptose Broth.

The American Public Health Association specifies using m Endo Broth MF in the standard total coliform membrane filtration procedure for testing water[2] and bottled water.[3] APHA also specifies using m Endo Broth MF in the delayed-incubation total coliform procedure by adding sodium benzoate to make m Endo preservative medium.[2] The coliform bacteria are defined as bacteria that produce a red colony with a metallic sheen within 24 hours incubation at 35°C on an Endo-type medium.

The U. S. Environmental Protection Agency specifies using m Endo Broth MF in the total coliform methods for testing water using single-step, two-step and delayed incubation membrane filtration methods.[4,5]

Principles of the Procedure

m Endo Broth MF contains Tryptose, Casitone and Thiopeptone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins, which stimulate bacterial growth. Lactose is the carbohydrate. Phosphates are buffering agents. Sodium Chloride maintains the osmotic balance of the medium. Sodium Desoxycholate and Sodium Lauryl Sulfate are added as inhibitors. Basic Fuchsin is a pH indicator. Sodium Sulfite is added to decolorize the Basic Fuchsin solution. Bacto Agar is the solidifying agent.

Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulfite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism produces aldehydes with the rapid fermentation of lactose. If the inoculum is too heavy, the sheen will be suppressed. Lactose non-fermenting bacteria form clear, colorless colonies.

Formula

m Endo Broth MF

Formula Per Liter

Bacto Yeast Extract	1.5 g
Bacto Casitone	5 g
Thiopeptone	5 g
Bacto Tryptose	10 g
Bacto Lactose	12.5 g
Sodium Desoxycholate	0.1 g
Dipotassium Phosphate	4.375 g
Monopotassium Phosphate	1.375 g
Sodium Chloride	5 g
Sodium Lauryl Sulfate	0.05 g
Sodium Sulfite	2.1 g
Bacto Basic Fuchsin	1.05 g

Final pH 7.2 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM

AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Liver, Thyroid

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

m Endo Broth MF

Materials Required but not Provided

95% Ethanol (Not denatured)
Glassware
Distilled or deionized water
Membrane filter apparatus
Membrane filters
Membrane filter absorbent pads
Petri dishes, 60 mm
Incubator (35°C)

Method of Preparation

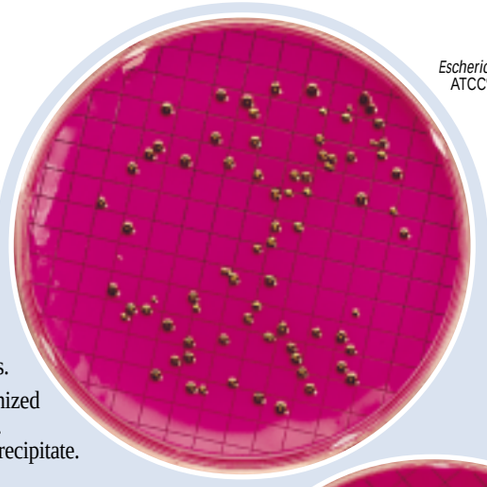
1. Suspend 48 grams in 1 liter distilled or deionized water containing 20 ml ethanol (95%, not denatured).
2. Boil to dissolve completely. **Do Not Autoclave**.

Specimen Collection and Preparation

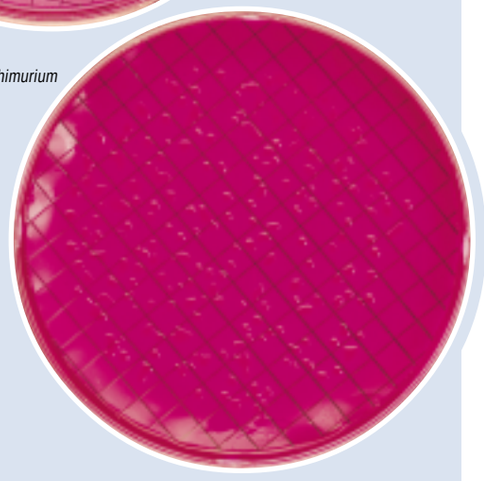
Collect samples and process according to recommended guidelines for enumerating coliforms in water.[2,3,4,5]

Test Procedure

1. Place a membrane filter absorbent pad inside a sterile 60 mm petri dish.
2. Add 1.8-2.0 ml m Endo Broth MF to each pad.
3. Filter the water sample through a membrane filter.
4. Place filter top side up on the pad using a rolling motion to avoid entrapping air bubbles.
5. Incubate at 35°C for 24 ± 2 hours.
6. Observe and count all colonies that are red and have a metallic sheen.



Escherichia coli
ATCC® 25922



Salmonella typhimurium
ATCC® 14028

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish purple, free-flowing, homogeneous.
Solution:	4.8% solution, soluble in distilled or deionized water containing 2% ethanol on boiling. Solution is pinkish-red, opalescent with precipitate.
Reaction of 4.8% Solution at 25°C:	pH 7.2 ± 0.1

Cultural Response

Prepare m Endo Broth MF per label directions. Use the membrane filter technique to inoculate filters. Incubate on pads saturated with m Endo Broth MF at 35 ± 2°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	20-80	good	red with green metallic sheen
Salmonella typhimurium	14028	20-80	good	colorless to pink
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Results

All colonies that are red and have the characteristic metallic sheen are considered coliforms. The sheen may cover the entire colony, may only be in the center or may appear only around the edges.

Limitations of the Procedure

1. Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies (dark red or nucleated colonies without sheen). It is advisable to verify both colony types.[2]

References

1. **Fifield, C. W., and C. P. Schaufus.** 1958. Improved membrane filter medium for the detection of coliform organisms. J. Amer. Water Works Assoc. **50:**193.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 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 (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Bordner, R., and J. Winter (ed).** 1978. Microbiological methods for monitoring the environment, water and wastes. 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.

Packaging

m Endo Broth MF	100 g	0749-15
	500 g	0749-17
	25 x 2 ml	0749-36

Bacto® Enteric Fermentation Base

Intended Use

Bacto Enteric Fermentation Base is used with added carbohydrate and indicator for differentiating microorganisms based on fermentation reactions.

Summary and Explanation

The fermentative properties of bacteria are valuable criteria in their identification.[1,2,3,4] A basal medium for determining the fermentation reactions of microorganisms must be capable of supporting growth of

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	1.8% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium plain + Andrede's Indicator:	Light pinkish amber, clear without precipitate.
Reaction of 1.8% Solution at 25°C:	pH 7.2 ± 0.1

Cultural Response

Prepare Enteric Fermentation Base per label directions, with addition of 1% Andrade's Indicator, with and without 1% dextrose. Inoculate each tube with one drop from an undiluted suspension of the test organism. Incubate at 35 ± 2°C for 18-24 hours. Acid production is indicated by a change in color from light amber to dark pink or red. Check for gas production in at least 3% of the volume of the fermentation vial.

ORGANISM	ATCC®	GROWTH	PLAIN ACID/GAS	w/ DEXTROSE ACID/GAS
Escherichia coli	25922*	good	–/–	+/+
Salmonella typhimurium	14028*	good	–/–	+/+
Shigella flexneri	12022*	good	–/–	+/–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Escherichia coli* ATCC® 25922 with Dextrose | *Escherichia coli* ATCC® 25922 plain

test organisms and be free from fermentable carbohydrates. Enteric Fermentation Base is prepared according to the formula described by Edwards and Ewing.[5,6]

Principles of the Procedure

Beef Extract and Peptone provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Sodium Chloride maintains the osmotic balance of the medium. The microorganisms tested are differentiated by their ability to ferment a particular carbohydrate that has been added to the Enteric Fermentation Base. The fermentation and resultant acid production are indicated by a change in color of the pH indicator (Andrade's indicator) which is also added to the Enteric Fermentation Base.

Formula

Enteric Fermentation Base

Formula Per Liter

Beef Extract	3 g
Peptone	10 g
Sodium Chloride	5 g

Final pH 7.2 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Enteric Fermentation Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Choice of filter-sterilized carbohydrate(s)
Andrade's Indicator (0.5 g Acid Fuchsin in 100 ml water plus 16 ml of 1.0 N Sodium Hydroxide)

Method of Preparation

1. Suspend 18 grams in 1 liter distilled or deionized water.
2. Add 10 ml Andrade's Indicator.
3. Heat to boiling to dissolve completely.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45 to 50°C in a waterbath.
6. Add appropriate amounts of sterile carbohydrates as indicated in the table below.

CARBOHYDRATE	FINAL CONCENTRATION	ADD BEFORE AUTOCLAVING	ADD AFTER AUTOCLAVING
Adonitol	0.5%	X	–
Arabinose	0.5%	–	X
Cellobiose	0.5%	–	X
Dextrose (Glucose)	1%	X	–
Dulcitol	0.5%	X	–
Glycerol*	0.5%	X	–
Inositol	0.5%	X	–
Lactose	1%	–	X
Mannitol	1%	X	–
Salicin	0.5%	X	–
Sucrose	1%	–	X
Xylose	0.5%	–	X

*Medium containing glycerol should be autoclaved for 10 minutes at 15 lbs pressure (121°C).

7. Dispense 3 ml amounts into test tubes containing inverted fermentation vials (Durham tubes).

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7,8,9]
2. Process each specimen, using procedures appropriate for that specimen or sample.[7,8,9]

Test Procedure

For a complete discussion on identification of *Enterobacteriaceae*, refer to the appropriate procedures outlined in the references.[5,6,10]

Results

A positive result for gas includes production in at least 3% of the volume of the fermentation tube. A positive reaction for gas production is a change in color from light amber to dark pink or red.

Limitations of the Procedure

1. Negative tubes remain colorless and should be observed regularly for a total of 30 days.

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc., St. Louis, MO.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.)**. 1995. Manual of clinical microbiology, 6th edition. ASM Press, Washington, D.C.
3. **Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams.** 1994. Bergey's manual of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, MD.
4. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition. Elsevier Science Publishing Co., Inc., New York, NY
5. **Edwards, P. R., and W. H. Ewing**. 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
6. **Balows, A., and W. J. Hausler**. 1981. Diagnostic Procedures for Bacteria, Mycotic and Parasitic Infections, 6th ed. American Public Health Association, Washington, D.C.

7. **Baron, E. J., and S. M. Finegold.** 1990. Bailey & Scott's Diagnostic Microbiology, 8th ed. C.V. Mosby Company, St. Louis, MO.
8. **Gilligan, P. H.** 1995. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
9. **Pezzlo, M. (ed.).** 1994. 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.
10. **Isenberg, H. D. (ed.).** 1992. Conventional tests (18 to 24 hours) - Carbohydrate Fermentation Test, p. 1.19.28. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology. Washington, D.C.

Packaging

Enteric Fermentation Base	500 g	1828-17

Bacto® m Enterococcus Agar

Intended Use

m Enterococcus Agar is used for isolating and enumerating enterococci in water and other materials by membrane filtration or pour plate technique.

Also Known As

m Enterococcus Agar is also referred to as m Azide Agar

Summary and Explanation

The enterococcus group is a subgroup of the fecal streptococci that include *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. avium*.[1] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10°C and 45°C.[1] The enterococci portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1] m Enterococcus Agar is used in standard methods for the detection of fecal streptococcus and enterococcus groups using the membrane filtration technique.[1]

m Enterococcus Agar was developed by Slanetz et al.[2] for the enumeration of enterococci by the membrane filtration technique. A modification of m Enterococcus Agar, adding triphenyltetrazolium chloride (TTC), was described by Slanetz and Bartley[3]. This modified medium proved to be a superior membrane filtration medium for the enumeration of enterococci. Increased recovery and larger colonies were obtained by incubating the inoculated membranes on the agar surface instead of on pads saturated with liquid medium. The membrane filtration method has the advantages of being simpler to perform, not requiring confirmation and permitting a direct count of enterococci in 48 hours. Burkwell and Hartman[4] added 0.2% sodium carbonate and 0.05% Tween® 80 to m Enterococcus Agar to increase the sensitivity for the direct plating method.

Principles of the Procedure

Tryptose 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 for the

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, very slightly to slightly opalescent, without significant precipitate.
Prepared Medium:	Light amber, slightly opalescent, without precipitate.
Reaction of 4.2% Solution at 25°C:	pH 7.2 ± 0.2

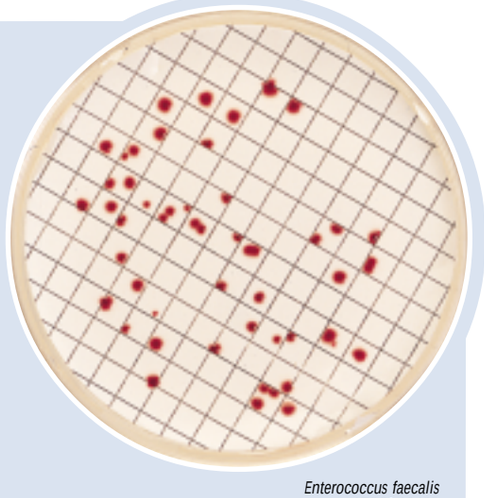
Cultural Response

Prepare m Enterococcus Agar per label directions. Inoculate medium using the membrane filter technique. Incubate in humid atmosphere inoculated medium at 35 ± 0.5°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY OF COLONY
Enterococcus faecalis	19433	20-60	good	pink to red
Enterococcus faecalis	29212*	20-60	good	pink to red
Escherichia coli	25922*	1,000	marked to complete inhibition	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol™ Disks Technical Information.



Enterococcus faecalis ATCC® 19433

medium. Sodium Azide is the selective agent to suppress the growth of gram negative organisms. Bacto Agar is the solidifying agent. Triphenyltetrazolium Chloride (TTC) is the dye used as an indicator of bacterial growth. TTC is reduced to the insoluble formazan inside the bacterial cell, resulting in the production of red colonies.

Formula

m Enterococcus Agar

Formula Per Liter

Bacto Tryptose	20 g
Bacto Yeast Extract	5 g
Bacto Dextrose	2 g
Dipotassium Phosphate	4 g
Sodium Azide	0.4 g
Bacto Agar	10 g
2,3,5-Triphenyl Tetrazolium Chloride	0.1 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

m Enterococcus Agar

Materials Required But Not Provided

Glassware
Sterile Petri dishes, 50 x 9 mm
Membrane filtration equipment
Incubator (35°C)
Fluorescent lamp
Magnifying lens
Distilled or deionized water

Method of Preparation

1. Suspend 42 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. DO NOT AUTOCLAVE.
4. Dispense into 9 x 50 mm Petri dishes to a depth of 4-5 mm (approximately 4-6 ml).

Specimen Collection and Preparation

Collect water samples as described in Standard Methods for the Examination of Water and Wastewater, Section 9230[1] or by laboratory policy.

Test Procedure

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in Standard Methods for the Examination of Water and Wastewater, Section 9230C.[1]
2. Choose a sample size so that 20-60 colonies will result.
3. Transfer the filter to agar medium in a Petri dish, avoiding air bubbles beneath the membrane.
4. Let plates stand for 30 minutes.
5. Invert plates and incubate at 35 ± 0.5°C for 48 hours.

Direct plating procedure

1. Inoculate medium with a specimen using the streak plate method.
2. Incubate plates at 35 ± 2°C for 24-48 hours.

Results[1]

Count all light and dark red colonies as enterococci. Count colonies using a fluorescent lamp and a magnifying lens.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Slanetz, Bent, and Bartley.** 1955. Public Health Rep. **70**:67.
3. **Slanetz, and Bartley.** 1957. J. Bacteriol. **74**:591.
4. **Burkwell, and Hartman.** 1964. Appl. Microbiol. **12**:18.

Packaging

m Enterococcus Agar	100 g	0746-15
	500 g	0746-17

Bacto® Eugon Agar
Bacto Eugon Broth

Intended Use

Bacto Eugon Agar and Eugon Broth are used for cultivating a wide variety of microorganisms, particularly in mass cultivation procedures.

Also Known As

Eugon media are also referred to as Eugonic Agar and Eugonic Broth.

User Quality Control

Identity Specifications

Eugon Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.54% solution, soluble in distilled or deionized water upon boiling. Light amber, slightly opalescent, precipitate may be visible.
Prepared Medium:	Light amber, slightly opalescent, precipitate may be visible.
Reaction of 4.54% Solution at 25°C:	pH 7.0 ± 0.2

Eugon Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.04% solution, soluble in distilled or deionized water upon boiling. Light amber, clear, may have a slight precipitate.
Prepared Medium:	Light amber, clear, may have a slight precipitate.
Reaction of 3.04% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepared Eugon Agar and Eugon Broth per label directions. Inoculate prepared medium and incubate for 18-48 hours (up to 72 hours if necessary). *Candida albicans* and *Aspergillus niger* should be incubated at 30 ± 2°C; all other cultures should be incubated at 35 ± 2°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	fair to good
Brucella abortus	4315	100-1,000	good
Candida albicans	26790	100-1,000	good
Lactobacillus fermentum	9338	100-1,000	good
Shigella flexneri	12022*	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

Eugon Agar and Eugon Broth are prepared according to the formula described by Vera.[1] Eugon media were developed to obtain eugonic (luxuriant) growth of fastidious microorganisms.[2] These formulations can be used with or without enrichment. Enriched with blood, Eugon media support the growth of pathogenic fungi including *Nocardia*, *Histoplasma* and *Blastomyces*. With the addition of Supplement B, excellent growth of *Neisseria*, *Francisella* and *Brucella* is achieved. The unenriched media support rapid growth of lactobacilli associated with cured meat products, dairy products and other food.

Niven[3] reported the use of Eugon Agar for the detection of lactic acid in cured meats, and recommended it for investigating spoilage in meats. Harrison and Hansen[4] employed the medium for plate counts of the intestinal flora of turkeys. Frank[5] showed its usefulness in germinating anaerobic spores pasteurized at 104°C.

Eugon Agar is specified in the Compendium of Methods for the Microbiological Examination of Food.[6]

Principles of the Procedure

Tryptose and Soytone provides the nitrogen, vitamins and amino acids in Eugon Agar and Eugon Broth. The high concentration of Dextrose is the energy source for rapid growth of bacteria. L-Cystine and Sodium Sulfite are added to stimulate growth. Sodium Chloride maintains the osmotic balance of the media. The high carbohydrate content along with high sulfur (cystine) content improves growth with chromogenicity.[2] Bacto Agar is the solidifying agent in Eugon Agar.

Formula

Eugon Agar

Formula Per Liter

Bacto Tryptose	15 g
Bacto Soytone	5 g
Bacto Dextrose	5.5 g
L-Cystine	0.7 g
Sodium Chloride	4 g
Sodium Sulfite	0.2 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Eugon Broth

Formula Per Liter

Bacto Tryptose	15 g
Bacto Soytone	5 g
Bacto Dextrose	5.5 g
L-Cystine	0.7 g
Sodium Chloride	4 g
Sodium Sulfite	0.2 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Eugon Agar
Eugon Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-55°C) (optional)
Sterile Petri dishes or Sterile tubes
5% sterile defibrinated blood (optional)
Bacto Supplement B (optional)

Method of Preparation

1. Suspend the appropriate amount of medium 1 liter distilled or deionized water:

Eugon Agar	45.4 g/l
Eugon Broth	30.4 g/l

2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. OPTIONAL: When an enriched medium is being prepared, cool to 50-55°C prior to adding the desired enrichment. After the enrichment is added, mix well.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion on bacteria and fungi from clinical specimens, refer to the appropriate procedures outlined in the references.[7,8] For the examination of bacteria and fungi in food refer to standard methods.[6,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Eugon Agar is not recommended as a blood agar base for hemolytic reactions because of its high sugar content.
3. It is suggested that Eugon Agar be prepared as required. Do not melt and resolidify media containing enrichments.

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 isolation-cultivation-identification-maintenance medical bacteria, p.301-303. vol. 1. 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 health 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. **70**:269.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
7. **Isenberg, H. D. (ed.),** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.),** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Eugon Agar	500 g	0589-17
Eugon Broth	500 g	0590-17

Bacto® m FC Agar
Bacto m FC Broth Base
Bacto Rosolic Acid

Intended Use

Bacto m FC Agar and Bacto m FC Broth Base are used with Bacto Rosolic Acid in cultivating and enumerating fecal coliforms by the membrane filter technique at elevated temperatures.

Also Known As

M-FC Medium

Summary and Explanation

Geldreich et al.[1] formulated a medium to enumerate fecal coliforms (MFC) using the membrane filter (MF) technique without prior enrichment. Fecal coliforms, i.e., those found in the feces of warm-blooded animals, are differentiated from coliforms from environmental sources by their ability to grow at 44.5 ± 0.5°C.[2]

Many Standard Methods membrane filtration procedures specify M-FC medium for testing water. The American Public Health Association (APHA) specifies M-FC medium and incubation at 44.5 ± 0.5°C in the fecal coliform membrane filter procedure, the delayed-incubation fecal coliform procedure, the two-layer agar method for recovering injured fecal coliforms,[2] and in the membrane filter method for fecal coliforms in bottled water.[3] The Association of Official Analytical Chemists (AOAC) specifies m-FC Agar for detecting total coliforms and fecal coliforms in foods.[4]

The U. S. Environmental Protection Agency specifies using M-FC medium in fecal coliform methods for testing water by the direct MF method or the delayed-incubation MF method.[5,6]

Principles of the Procedure

m FC Agar and m FC Broth Base contain Tryptose and Proteose Peptone No. 3 as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Lactose is a carbohydrate. Bile Salts No. 3 inhibits growth of gram-positive bacteria. m FC Agar contains Bacto Agar as the solidifying agent. The differential indicator system combines Aniline Blue and Rosolic Acid.

Colonies of fecal coliforms are blue; non-fecal coliforms and other organisms are gray to cream-colored.

Formula

m FC Agar

Formula Per Liter

Bacto Tryptose	10 g
Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	12.5 g
Bacto Bile Salts No. 3	1.5 g
Sodium Chloride	5 g
Bacto Agar	15 g
Aniline Blue	0.1 g

Final pH 7.4 ± 0.2 at 25°C

m FC Broth Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Sodium Chloride	5 g
Bacto Lactose	12.5 g
Bacto Bile Salts No. 3	1.5 g
Aniline Blue (Water Blue)	0.1 g

Final pH 7.4 ± 0.2 at 25°C

Rosolic Acid

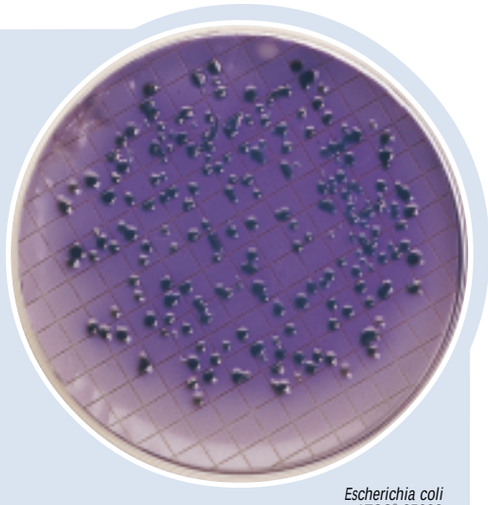
Rosolic Acid	1 g/vial

User Quality Control

Identity Specifications

m FC Agar

Dehydrated Appearance:	Beige with slight blue tint, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling. Without 1% rosolic acid: blue, very slightly to slightly opalescent, may have a slight precipitate. With 1% rosolic acid: cranberry red, slightly opalescent, may have a slight precipitate.
Prepared Medium:	Without 1% rosolic acid: blue, slightly opalescent. With 1% rosolic acid: cranberry red, slightly opalescent.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2 (without 1% rosolic acid)



Escherichia coli ATCC® 25922

m FC Broth Base

Dehydrated Appearance:	Beige with slight blue tint, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water on boiling. Without 1% rosolic acid: blue, slightly opalescent, may have a very fine precipitate. With 1% rosolic acid: cranberry red, slightly opalescent, may have a very fine precipitate.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2 (without 1% rosolic acid)

Rosolic Acid

Dehydrated Appearance:	Dark reddish-brown with metallic green particles, free-flowing, fine crystalline powder.
Solution:	1.0% solution, soluble in 0.2 N NaOH. Solution is deep red, clear to very slightly opalescent.

Cultural Response

m FC Agar and m FC Broth Base

Prepare mFC Agar and mFC Broth Base per label directions with 1% Rosolic Acid. Using the membrane filter technique, inoculate and incubate plates at 44.5 ± 0.5°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	19433*	1,000-2,000	markedly to completely inhibited	–
Escherichia coli	25922*	20-80	good	blue

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. Rosolic Acid: **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Rosolic Acid at 15-30°C. Rehydrated Rosolic Acid (1% solution) is stable for 2 weeks if stored at 2-8°C in the dark.

Expiration Date

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

Procedure

Materials Provided

m FC Agar
m FC Broth Base
Rosolic Acid

Materials Required but not Provided

0.2 N sodium hydroxide
1 N hydrochloric acid
Glassware
Distilled or deionized water
Waterproof plastic bags
Waterbath (35°C)
Waterbath (44.5 ± 0.5°C)

Method of Preparation

Rosolic Acid

1. Prepare a 1% solution, dissolving 1 gram in 100 ml 0.2 N NaOH.

m FC Agar

1. Suspend 52 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 10 ml of a 1% solution of Rosolic Acid in 0.2 N NaOH.
4. Continue heating for 1 minute. Do Not Autoclave.
5. If necessary, adjust to pH 7.4 with 1 N HCl.

m FC Broth Base

1. Suspend 3.7 grams in 100 ml distilled or deionized water.
2. Add 1 ml of a 1% solution of Rosolic Acid in 0.2 N NaOH.
3. If necessary, adjust to pH 7.4 with 1 N HCl.
4. Heat to boiling.
5. Cool before dispensing.

Specimen Collection and Preparation

Collect samples and process according to recommended guidelines.[2-6]

Test Procedure

m FC Agar

1. Filter duplicate samples through separate membrane filters.
2. Transfer the filters to the surface of separate mFC Agar plates.
3. Place each plate in a separate waterproof plastic bags Submerge in different waterbaths, one set at 35 ± 2°C and one set at 44.5 ± 0.5°C; incubate for 24 ± 2 hours.
4. Incubate one set of plates at 35°C and one set at 44.5 ± 0.5°C for 24 ± 2 hours.

Results

Colonies of fecal coliforms will be various shades of blue. Non-fecal coliforms are gray to cream-colored.

Limitations of the Procedure

1. A few nonfecal coliform colonies may be observed on m FC media due to the selective action of the elevated temperature and the addition of the Rosolic Acid. It may be useful to elevate the temperature to 45 ± 0.2°C to eliminate *Klebsiella* strains from the fecal coliform group.[2]

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 (ed.).** 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 (ed.). 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 (ed).** 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.

Packaging

m FC Agar	100 g	0677-15
	500 g	0677-17
m FC Broth Base	100 g	0883-15
	500 g	0833-17
Rosolic Acid	6 x 1 g	3228-09

Bacto® m FC Basal Medium

Intended Use

Bacto m FC Basal Medium is used with MUG or BCIG for cultivating and enumerating fecal coliforms by the membrane filter technique at elevated temperatures.

Summary and Explanation

Ciebin et al.[1] described a modification of m FC Medium called FC Basal Medium, in which the chromogenic substrate 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (BCIG) is added for quantitative recovery of *Escherichia coli* from untreated water samples to show fecal contamination using membrane filter methods.

Standard method procedures use media with the fluorogenic substrate, 4-methylumbelliferyl β-D-glucuronide (MUG) to enumerate *E. coli* by membrane filter methods.[2] Disadvantages of using MUG include the requirement of ultra violet light, possible diffusion of fluorescence from the colony to the surrounding medium and background fluorescence of membrane filters.[3] Using BCIG in place of MUG to detect β-glucuronidase activity, gives visible blue colonies and an indigo-blue complex that remains within the colony. Ciebin et al.[1] found FC-BCIG Medium comparable to standard MUG-based media for detection of ß-glucuronidase activity of *E. coli*.

In another study, Ciebin et al.[4] formulated DC Medium using FC Basal Medium supplemented with lactose, BCIG and cefsulodin. It is a differential coliform medium for the enumeration of coliforms and *E. coli* in potable water using membrane filtration. Ciebin et al. compared DC Medium to LES Endo Medium and FC-BCIG Medium. They found DC Medium superior to LES Endo Medium in recovering coliforms and equivalent to FC-BCIG Medium in recovering *E. coli*.

Principles of the Procedure

m FC Basal Medium contains Tryptose and Proteose Peptone No. 3 as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Bile Salts No. 3 inhibits the growth of gram-positive microorganisms. Bacto Agar is the solidifying agent.

Formula

m FC Basal Medium

Formula Per Liter

Bacto Tryptose	10 g
Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Bile Salts No. 3	1.5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent, may have slight precipitate.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

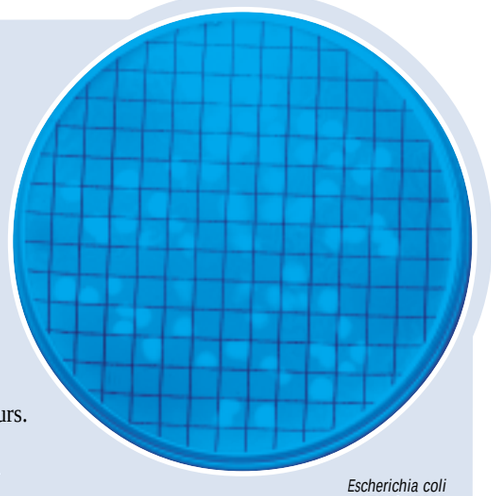
Cultural Response

Prepare m FC Basal Medium per label directions, with the addition of 0.01% MUG. Using membrane filter technique, inoculate and incubate at 44.5 ± 0.5°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	19433*	300-1,000	markedly to completely inhibited	–
Escherichia coli	25922*	30-200	good	blue-white fluorescence

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Escherichia coli ATCC® 25922

Procedure

Materials Provided

m FC Basal Medium

Materials Required but not Provided

MUG (4-methylumbelliferyl b-D-glucuronide)
BCIG (5-bromo-6-chloro-3-indolyl b-D-glucuronide)
1 N HCl
Glassware
Membrane filtration apparatus
Petri dishes, 60 mm
Distilled or deionized water
Waterproof plastic bags
Waterbath (35°C)
Waterbath (44.5°C)

Method of Preparation

1. Suspend 39.5 grams in 1 liter distilled or deionized water.
2. Add 100 mg MUG or BCIG, as desired, and boil to dissolve completely. **Do Not Autoclave.**
3. If necessary, adjust to pH 7.4 with 1 N HCl.
4. Dispense into 60 mm sterile petri dishes.

Specimen Collection and Preparation

Collect samples and process according to recommended guidelines for water testing.[2]

Test Procedure

1. Filter duplicate water samples through membrane filtration apparatus.
2. Transfer each membrane to the surface of the m FC Basal Medium plate. Place cultures in waterproof plastic bags. Submerge petri dishes in waterbath. Incubate one set of plates at 35 ± 2°C and one set at 44.5 ± 0.5°C for 24 ± 2 hours.
3. Count number of colonies and record observations. m FC-MUG plates are read under long-wavelength (366 nm) ultraviolet light.

Results

m FC-MUG

β-glucuronidase-positive organisms produce a blue-white fluorescence.

β-glucuronidase-positive organisms do not fluoresce.

m FC-BCIG

β-glucuronidase-positive organisms produce a visible blue colony.

β-glucuronidase-positive organisms produce a non-blue colony.

References

1. **Ciebin, B. W., M. H. Brodsky, R. Eddington, G. Horsnell, A. Choney, G. Palmateer, A. Ley, R. Joshi, and G. Shears.** 1995. Comparative evaluation of modified m-FC and m-TEC medium for membrane filter enumeration of *Escherichia coli* in water. Appl. Environ. Microbiol. **61**:3940-3942.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Farber, J. M.** 1986. Potential use of membrane filters and a fluorogenic reagent-based solid medium for the enumeration of Escherichia coli in foods. Can. Inst of Food Sci. and Tech. J. **19**:34-37.
4. **Ciebin, B. W., R. N. Schop, and M. H. Brodsky.** 1997. DC medium: a differential coliform medium for simultaneous enumeration of coliforms and *Escherichia coli* in water by membrane filtration. Q-152. Abstr. 97th Annu. Meet. Am. Soc. for Microbiol. 1997. American Society for Microbiology, Washington, D.C.

Packaging

m FC Basal Medium	500 g	0698-17

Bacto® Fildes Enrichment

User Quality Control

Identity Specifications

Solution Appearance: Dark brown solution.

Cultural Response

Prepare Heart Infusion Agar per label directions. Aseptically add 5% Fildes Enrichment to the cooled medium at 50-55°C and pour plates. Inoculate and incubate at 35 ± 2°C under increased CO_2 for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Haemophilus influenzae	19418	100-1,000	good
Haemophilus parainfluenzae	7901	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Intended Use

Bacto Fildes Enrichment is used to enrich media for cultivating *Haemophilus influenzae*.

Also Known As

Fildes Enrichment is also referred to as Fildes Broth.

Summary and Explanation

Fildes Enrichment, a sterile digest of sheep blood, is prepared according to Fildes.[1,2] This enrichment is recommended for use in liquid and solid media for culturing *Haemophilus influenzae* and other fastidious microorganisms requiring blood derivatives for optimal growth.

Body fluid specimens likely to harbor *Haemophilus* species should be inoculated on 5% sheep blood agar, chocolate agar and a suitable enrichment broth, such as Fildes Broth.[3] Fildes-enriched gonococcal medium, composed of gonococcal agar base containing 5% Fildes Enrichment, 5% horse blood and 3 µg of vancomycin per ml, is used for lymph node aspirates and lesion scrapings from patients with suspected chancroid.[3]

Principles of the Procedure

Fildes Enrichment is a rich source of factors that will promote growth of fastidious microorganisms. The growth factors include hemin (X factor) and nicotinamide adenine dinucleotide (NAD or V factor) required by *H. influenzae* and other *Haemophilus* species.

Formula

Fildes Enrichment

A sterile digest of sheep blood.

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper, established laboratory procedure in handling and disposing of infectious materials.

Storage

Store Fildes Enrichment at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Fildes Enrichment

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Fildes Enrichment is a ready-to-use solution. Many factors in Fildes Enrichment are heat labile. This enrichment cannot be heated and must be added aseptically in the proper amounts to media that have been sterilized in the autoclave and cooled to 50-55°C.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy or appropriate references.[3,4]

Test Procedure

Fildes Enrichment is usually employed in prepared media at a final concentration of 5% for optimal results. Some formulas may require higher or lower concentrations. Add Fildes Enrichment as required.

Body fluids and other clinical specimens inoculated in Fildes Enrichment should be incubated for 7 days at 35-37°C.[3]

Results

Carefully examine clinical specimens incubated in Fildes Enrichment for evidence of growth.[3]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly with this enrichment.
2. *Haemophilus* species cannot be depended on to impart obvious turbidity to broths even when they are present at densities exceeding 10^9 CFU/ml.[3]

References

1. **Fildes.** 1920. Br. J. Exp. Pathol. **1**:129-130.
2. **Fildes.** 1921. Br. J. Exp. Pathol. **2**:16-25.
3. **Campos, J. M.** 1995. *Haemophilus*, p. 556-565. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Fildes Enrichment	6 x 5 ml	0349-57
	100 ml	0349-72
	6 x 100 ml	0349-73

Bacto® Fish Peptone No. 1

Intended Use

Bacto Fish Peptone No. 1 is used in preparing microbiological culture media.

Summary and Explanation

Fish Peptone No. 1 is a non-bovine origin peptone. Fish Peptone No. 1 was developed by Difco Laboratories for pharmaceutical and vaccine production to reduce Bovine Spongiform Encephalopathy (BSE) risk. Fish Peptone No. 1 may substitute for other peptones, depending on organism and production application.

Principles of the Procedure

Fish Peptone No. 1 is a non-mammalian/animal peptone used as a nitrogen source in microbiological culture media.

Typical Analysis

Physical Characteristics

Ash (%)	34.8	Loss on Drying (%)	3.4
Clarity, 1% Soln (NTU)	0.9	pH, 1% Soln	6.9
Filterability (g/cm^2)	3.4		

Carbohydrate (%)

Total	<0.1

Nitrogen Content (%)

Total Nitrogen	10.6	AN/TN	30.2
Amino Nitrogen	3.2		

Amino Acids (%)

Alanine	3.48	Lysine	2.51
Arginine	2.19	Methionine	0.83
Aspartic Acid	3.21	Phenylalanine	0.95
Cystine	0.24	Proline	2.19
Glutamic Acid	5.27	Serine	1.27
Glycine	5.29	Threonine	1.17
Histidine	1.54	Tryptophan	0.15
Isoleucine	0.92	Tyrosine	0.45
Leucine	2.16	Valine	1.43

Inorganics (%)

Calcium	0.020	Phosphate	3.848
Chloride	9.326	Potassium	4.183
Cobalt	<0.001	Sodium	9.351
Copper	0.001	Sulfate	1.004
Iron	0.003	Sulfur	1.629
Lead	<0.001	Tin	<0.001
Magnesium	0.017	Zinc	0.002
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.3	PABA	95.0
Choline (as Choline Chloride)	4170.7	Pantothenic Acid	63.2
Cyanocobalamin	0.3	Pyridoxine	7.2
Folic Acid	1.5	Riboflavin	26.8
Inositol	2820.0	Thiamine	NA
Nicotinic Acid	603.0	Thymidine	55.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	18
Salmonella	negative	Thermophile Count	379

User Quality Control

Identity Specifications

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 1% solution is light to medium amber, clear to very slightly opalescent, may have a slight precipitate.

Reaction of 1% Solution at 25°C: pH 6.7 ± 0.2

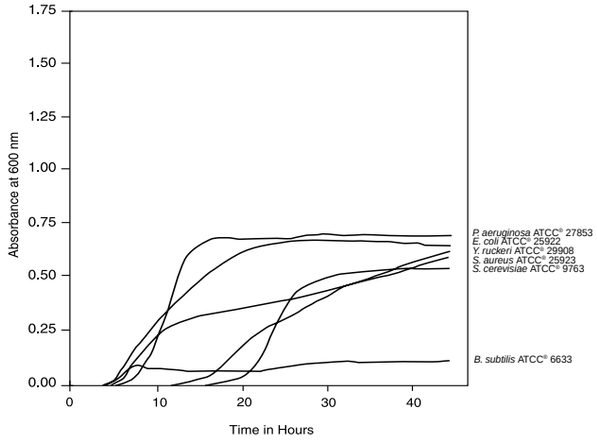
Cultural Response

Prepare a 1% concentration of Fish Peptone No. 1 with the addition of 0.5% sodium chloride. Inoculate test organisms and incubate for 18-48 hours at 35 ± 2°C. Incubate *Vibrio tubiashii* for 18-48 hours at 25 ± 2°C. *Saccharomyces cerevisiae* is tested with the addition of 0.5% dextrose. *Vibrio tubiashii* is tested with the addition of 1.5% sodium chloride.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis†	6633	100-1,000	fair to good
Escherichia coli	25922*	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good
Vibrio tubiashii	19105	100-1,000	good

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

†*Bacillus subtilis* is available as Bacto Subtilis Spore Suspension.



Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated product below 30°C. The dehydrated product is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Fish Peptone No. 1

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of peptone in the formula of the medium being prepared. Add Fish Peptone No. 1 as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures on the medium being prepared or the sample being analyzed.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on prepared medium.

Packaging

Fish Peptone No. 1	500 g	0551-17
	10 kg	0551-08

Bacto® Fletcher Medium Base

Intended Use

Bacto Fletcher Medium Base is used with sterile normal rabbit serum for isolating and cultivating *Leptospira*.

Summary and Explanation

In 1816, Adolf Weil described the first recognized infections of Leptospirosis in humans.[5] These cases were caused by *Leptospira interogans* serovar *icterohaemorrhagiae* and the disease was subsequently named Weil's Disease.[5] Leptospirosis is a zoonotic disease, having its reservoir in wild, domestic, and peridomestic animals.[6] Infection usually results from direct or indirect exposure to the urine of leptospiruric animals.[6] Indirect exposure through contaminated water and soil accounts for most sporadic cases.[3] Direct exposure occurs in pet owners, veterinarians and persons working with livestock.[3]

Leptospirosis is typically a biphasic illness.[3,8] The infection is acute in onset, with a flu- like syndrome persisting for 4 to 7 days.[4] Onset of a second "immune" phase, in which meningitis, skin rash, and hepatic and renal involvement may be present, occurs within a few days.[4]

Fletcher Medium Base is prepared according to the formulation of Fletcher.[1] Myers et al.[2] reported Fletcher Medium, with a basal agar layer containing charcoal, to be superior to the standard medium for the maintenance of leptospiral cultures. Fletcher Medium Base prepared with sterile normal rabbit serum is specified for the isolation of *Leptospira*.[3,4,7]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.5 g in 920 ml distilled or deionized water, soluble upon boiling. Solution is very light amber, clear to very slightly opalescent without significant precipitate.
Prepared Medium:	Very light amber, very slightly to slightly opalescent without significant precipitate.
Reaction of 2.5 g in 920 ml distilled water:	pH 7.9 ± 0.1 at 25°C

Cultural Response

Prepare Fletcher Medium Base per label directions. Enrich with sterile normal rabbit serum. Inoculate and incubate at 30 ± 2°C for up to 5 days.

ORGANISM	ATCC®	INOCULUM	GROWTH
Leptospira interrogans serovar *australis*	23605	2-3 drops	good
Leptospira interrogans serovar *canicola*	23470	2-3 drops	good
Leptospira kirschneri serovar *grippotyphosa*	23604	2-3 drops	good

The cultures listed are the minimum that should be used for performance testing.

Principles of the Procedure

Bacto Peptone and Beef Extract provide the nitrogen, vitamins, carbon and amino acids in Fletcher Medium Base. Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent.

Sterile normal rabbit serum is added to the formula to stimulate growth of *Leptospira*.

Formula

Fletcher Medium Base

Formula Per Liter

Bacto Peptone	0.3 g
Bacto Beef Extract	0.2 g
Sodium Chloride	0.5 g
Bacto Agar	1.5 g

Final pH 7.9 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. *Leptospira* species are BioSafety Level 2 pathogens. Handling clinical specimen material potentially infected with *Leptospira* species should be performed in a Class II biological safety cabinet (BSC).[7]

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Fletcher Medium Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (56°C)
Sterile normal rabbit serum

Method of Preparation

1. Suspend 2.5 grams in 920 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 56°C.
4. Aseptically add 80 ml sterile normal rabbit serum at 56°C. Mix well.
5. Determine pH. If necessary, aseptically adjust to pH 7.9 ± 0.1 with 1 N HCl or 1 N NaOH.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy. Blood, cerebrospinal fluid (CSF) and

urine are the specimens of choice for the recovery of leptospires from patients with leptospirosis.[3]

Test Procedure[7]

1. Aseptically dispense into sterile screw-cap tubes in 5-7 ml amounts. Store at room temperature overnight.
2. Inactivate the whole medium the day following its preparation by placing the tubes in a water bath at 56°C for 1 hour.
3. Allow the medium to cool before inoculation.
4. Growth is first seen in approximately 10 days at 35°C (2 to 4 weeks at 25°C) as a cloud of minute granules that develop into microcolonies just below the surface.
5. Gram stain is not satisfactory. The microcolonies can be fixed with methanol and stained with Giemsa stain to show rod forms at the edges.[9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Fletcher, W.** 1928. Recent work on leptospirosis, tsutsugamushi disease and tropical typhus in the federated Malay States. Trans. Roy. Soc. Trop. Med. Hyg. **21**: 265-287.
2. **Myers, D. M., V. M. Varela-Diaz, and A. A. Siniuk.** 1973. Long-term survival of Leptospira in a biphasic culture medium containing charcoal. Am. Soc. Microbiol. **25**:514-516.
3. **Kaufmann, A. F., and R. S. Weyant**. 1995. *Leptospiraceae*, p. 621-625. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Koneman, E. W., S. D. Allen, V. R. Dowell, Jr., W. M. Janda, H. M. Sommers, and W. C. Winn, Jr.** 1988. Color atlas and textbook of diagnostic microbiology, 3rd ed. J. B. Lippincott Company, Philadelphia, PA.
5. **Elliott, S. H.** 1980. Discussion and clinical diagnosis of Leptospirosis. J. Am. Med. Tech. **42**:37-44.
6. **Faine, S. (ed.).** 1982. Guidelines for the control of Leptospirosis. W. H. O. Offset publication no. 67. World Health Organization, Geneva.
7. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
8. **Kelley, P. W.** 1992. Leptospirosis, p. 1295-1301. *In* S. L. Gorbach, J. G. Bartlett, and N. R. Blacklow (ed.), Infectious diseases. The W. B. Saunders Co., Philadelphia, PA.
9. **Weinman, D.** 1981. Bartonellosis and anemias associated with bartonella-like structures, p. 235-248. *In* A. Balows, and W. J. Hausler, Jr. (ed.), Diagnostic procedures for bacterial, mycotic and parasitic infections, 6th ed. American Public Health Association, Washington, D.C.

Packaging

Fletcher Medium Base	500 g	0987-17

Bacto® Folic AOAC Medium

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	5.50% solution (single strength) and 11.0% solution (double strength), soluble in distilled or deionized water on boiling.
	Solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	Very light amber, clear.
Reaction of 5.50% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Prepare Folic AOAC Medium per label directions. This medium should support the growth of *E. hirae* ATCC® 8043 when prepared in single strength and supplemented with folic acid.

Intended Use

Bacto Folic AOAC Medium is used for determining folic acid concentration by the microbiological assay technique.

Also Known As

AOAC is an abbreviation for Association of Official Analytical Chemists.

Summary and Explanation

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

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: to permit quantitation of the vitamin under test.

Folic AOAC Medium is prepared for use in the microbiological assay of folic acid according to the procedures of the Folic Acid Assay in AOAC.[1] *Enterococcus hirae* ATCC® 8043 (*Streptococcus faecium*) is the test organism in this assay.

Principles of the Procedure

Folic Acid AOAC Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *E. hirae* ATCC® 8043. The addition of folic acid in specified

increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Folic AOAC Medium

Formula Per Liter		
Bacto Vitamin Assay Casamino Acids	10	g
L-Asparagine	0.6	g
L-Tryptophane	0.2	g
L-Cysteine Hydrochloride	0.76	g
Bacto Dextrose	40	g
Adenine Sulfate	10	mg
Guanine Hydrochloride	10	mg
Uracil	10	mg
Xanthine	20	mg
p-Aminobenzoic Acid	1	mg
Pyridoxine Hydrochloride	4	mg
Thiamine Hydrochloride	400	µg
Calcium Pantothenate	800	µg
Nicotinic Acid	800	µg
Biotin	20	µg
Riboflavin	1	mg
Glutathione	5.2	mg
Sorbitan Monooleate Complex	0.1	g
Sodium Citrate	52	g
Dipotassium Phosphate	6.4	g
Magnesium Sulfate	0.4	g
Manganese Sulfate	20	mg
Sodium Chloride	20	mg
Ferrous Sulfate	20	mg

Final pH 6.7 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware is heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
4. Take precautions to keep sterilizing and cooling conditions uniform throughout the assay.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Folic AOAC Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Enterococcus hirae* ATCC® 8043
Sterile tubes
Distilled or deionized water
Folic Acid
0.01 N NaOH
Dilute HCl
Spectrophotometer or Nephelometer

Method of Preparation

1. Suspend 11 grams in 100 ml distilled or deionized water.
2. Heat to boiling for 2-3 minutes.
3. Distribute 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Follow assay procedures as outlined in AOAC.[1] It is essential that a standard curve be set up for each separate assay. Autoclaving and incubation conditions that can influence the standard curve readings cannot always be duplicated. The standard curve is obtained by using folic acid at levels of 0.0, 1, 2, 4, 6, 8 and 10 ng per assay tube (10 ml). Folic AOAC Medium may be used for both turbidimetric and titrimetric analysis. Turbidimetric readings should be taken after incubation at 35-37°C for 16-18 hours. Titrimetric determinations are best made following incubation at 35-37°C for 72 hours.

The folic acid required for the preparation of the standard curve may be prepared as follows:

A. Dissolve 50 mg dried folic acid in about 30 ml 0.01N NaOH and 300 ml distilled water.

B. Adjust the pH reaction to 7.5 ± 0.5 with diluted HCl solution. Dilute to 500 ml with distilled water.

C. Add 2 ml of the solution to 50 ml distilled water. Adjust the pH reaction to 7.5 ± 0.5. Dilute to 100 ml with distilled water. This yields a stock solution containing 2 mcg folic acid per ml.

D. Prepare the stock solution fresh daily.

The standard solution for the assay is made by diluting 1 ml of this stock solution to 1 liter with distilled water. This solution contains 2 ng folic acid per ml. Use 0.0, 0.5, 1, 2, 3, 4, and 5 ml per assay tube.

Some laboratories may wish to alter the concentration of folic acid recommended above for the standard curve. This is permissible if the concentration used is within the limits specified by AOAC.[1]

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.

2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

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

References

1. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC international, 16th ed. AOAC International, Arlington, VA.

Packaging

Folic AOAC Medium	100 g	0967-15*

*Store at 2-8°C

Bacto® Folic Acid Assay Medium

Intended Use

Bacto Folic Acid Assay Medium is used for determining folic acid concentration by the microbiological assay technique.

Summary and Explanation

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

1. Maintenance Medium: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Medium: To condition the test culture for immediate use.
3. Assay Medium: To permit quantitation of the vitamin under test.

Folic Acid Assay Medium is used in the microbiological assay of folic acid with *Enterococcus hirae* ATCC® 8043 as the test organism. Folic Acid Assay Medium is prepared according to the formula described by Capps, Hobbs and Fox,[1] modified with sodium citrate instead of sodium acetate.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off white to very light beige, free-flowing, homogeneous.
Solution:	3.75% (single strength) or 7.5% (double strength) solution, soluble in distilled or deionized water upon boiling for 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	Very light amber, clear, may have a very slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare single-strength Folic Acid Assay Medium per label directions. The medium should support the growth of *E. hirae* ATCC® 8043. The most effective range is 2-10 ng folic acid per 10 ml tube.

Principles of the Procedure

Folic Acid Assay Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *E. hirae* ATCC® 8043. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Folic Acid Assay Medium

Formula Per Liter	
Bacto Vitamin Assay Casamino Acids	12 g
Bacto Dextrose	40 g
Sodium Citrate	20 g
L-Cystine	0.2 g
DL-Tryptophane	0.2 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Thiamine Hydrochloride	2 mg
Pyridoxine Hydrochloride	4 mg
Riboflavin	2 mg
Niacin	2 mg
p-Aminobenzoic Acid	200 µg
Biotin	0.8 µg
Calcium Pantothenate	400 µg
Dipotassium Phosphate	1 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small

amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.

4. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Folic Acid Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Enterococcus hirae* ATCC® 8043
Sterile tubes
Sterile 0.85% saline
Distilled or deionized water
0.01 N NaOH
Dilute HCl
Folic Acid USP
Lactobacilli Agar AOAC
Lactobacilli Broth AOAC
Centrifuge
Spectrophotometer

Method of Preparation

1. Suspend 7.5 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Prepare assay samples according to references given in the specific assay procedures. Dilute the samples to approximately the same concentration as the standard solution.

Test Procedure

Prepare stock cultures of *E. hirae* ATCC® 8043 by stab inoculation of Lactobacilli Agar AOAC. Incubate at 35-37°C for 24-48 hours. Store tubes in the refrigerator. Make transfers at monthly intervals. Prepare the inoculum for assay by subculturing a stock culture of *E. hirae* ATCC® 8043 into a tube containing 10 ml of Lactobacilli Broth AOAC. After incubation at 35-37°C for 18-24 hours, centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 ml of sterile 0.85% saline. After the third wash, dilute the cell suspension 1:100 with sterile 0.85% saline. Use one drop of this latter suspension to inoculate each of the assay tubes.

It is essential that a standard curve be set up for each separate assay. Autoclaving and incubation conditions that influence the standard curve readings cannot always be duplicated. The standard curve is obtained by using folic acid at levels of 0.0, 2, 4, 6, 8 and 10 ng per 10 ml assay tube. Turbidimetric readings should be made after incubation at 35-37°C for 18-24 hours. Refrigerate tubes for 15-30 minutes to stop growth before reading.

Prepare the folic acid stock solution required for the standard curve as follows:

1. Dissolve 50 mg dried Folic Acid USP Reference Standard or equivalent in about 30 ml of 0.01 N NaOH and 300 ml distilled water.
2. Adjust to pH 7.5 ± 0.5 with diluted HCl solution. Add distilled water to give a volume of 500 ml.
3. Add 2 ml of the solution from step 2 to 50 ml distilled water. Adjust the pH to 7.5 ± 0.5 with HCl solution. Dilute to 100 ml with distilled water to give a stock solution containing 2 mcg folic acid per ml. Prepare the stock solution fresh daily.

Prepare the standard solution for the assay by diluting 1 ml of this stock solution in 1 liter with distilled water. This solution contains 2 ng folic acid per ml. Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml per assay tube.

Following incubation, place the tubes in the refrigerator for 15-30 minutes to stop growth. The growth can be measured by a turbidimetric method and the curve constructed from the values obtained. The most effective assay range is between the levels of 2 and 10 ng folic acid per 10 ml tube.

Results

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

Limitations of the Procedure

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

References

1. **Capps, Hobbs, and Fox.** 1948. J. Bacteriol. **55**:869.

Packaging

Folic Acid Assay Medium	100 g	0318-15

Bacto® Folic Acid Casei Medium
Bacto Folic Buffer A, Dried

Intended Use

Bacto Folic Acid Casei Medium is used for determining folic acid concentration by the microbiological assay technique.

Bacto Folic Buffer A, Dried is prepared for use with Folic Acid Casei Medium in the microbiological assay of serum folic acid.

Summary and Explanation

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

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

Folic Acid Casei Medium is prepared for the microbiological assay of folic acid, particularly folic acid in serum. *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469 is used as the test organism in this assay. Folic Acid Casei Medium is prepared according to the formulation described by Flynn, Williams, O'Dell and Hogan[1] and modified by Baker et al.[2] and Waters and Mollin.[3]

Total serum folic acid activity can vary depending on the disease state. It has been reported that normal subjects have a mean serum folic acid level of 9.9 ng per ml. Patients with uncomplicated pernicious anemia have a mean serum folic acid level of 16.6 ng per ml while patients with megaloblastic anemia have levels less than 4.0 ng per ml.

Folic Buffer A, Dried is used for preparing both the standard and the serum specimen in the microbiological assay of folic acid.

User Quality Control

Identity Specifications

Folic Acid Casei Medium

Dehydrated Appearance:	Off-white, homogeneous, with a tendency to clump.
Solution:	4.7% (single strength) and 9.4% (double strength) solution, soluble in distilled or deionized water upon boiling 1-2 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	Single-strength solution is very light amber, clear, may have a very slight precipitate.
Reaction of 4.7% Solution at 25°C:	6.7 ± 0.1

Folic Buffer A, Dried

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	1.54% solution, soluble in distilled or deionized water.
Solution Appearance:	Colorless to very light amber, clear.
Reaction of 1.54% Solution at 25°C:	pH 6.1 ± 0.05

Cultural Response

Prepare Folic Acid Casei Medium per label directions. The medium is tested by creating a standard curve using Folic Acid at concentrations of 0 to 1.0 ng per 10 ml. This medium should support the growth of *L. casei* subsp. *rhamnosus* ATCC® 7469 when prepared in single strength and supplemented with ascorbic acid and Folic Acid.

Principles of the Procedure

Folic Acid Casei Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. casei* subsp. *rhamnosus* ATCC® 7469. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Folic Acid Casei Medium

Formula Per Liter

Charcoal Treated Casitone	10 g
Bacto Dextrose	40 g
Sodium Acetate	40 g
Dipotassium Phosphate	1 g
Monopotassium Phosphate	1 g
DL-Tryptophane	0.2 g
L-Asparagine	0.6 g
L-Cysteine Hydrochloride	0.5 g
Adenine Sulfate	10 mg
Guanine Hydrochloride	10 mg
Uracil	10 mg
Xanthine	20 mg
Sorbitan Monooleate Complex	0.1 g
Glutathione (reduced)	5 mg
Magnesium Sulfate, Anhydrous	0.2 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	15 mg
Riboflavin	1 mg
p-Aminobenzoic Acid	2 mg
Pyridoxine Hydrochloride	4 mg
Thiamine Hydrochloride	400 µg
Calcium Pantothenate	800 µg
Nicotinic Acid	800 µg
Biotin	20 µg

Final pH 6.7 ± 0.1

Folic Buffer A, Dried

Formula Per Liter

Monopotassium Phosphate	10.656 g
Dipotassium Phosphate	3.744 g
Ascorbic Acid	1 g

Final pH 6.1 ± 0.05

Precautions

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

Storage

Store Folic Acid Casei Medium and Folic Buffer A, Dried at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Folic Acid Casei Medium
Folic Buffer A, Dried

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469
Ascorbic acid (25 mg)
Sterile 0.85% saline
Distilled and deionized water
0.01 N NaOH
0.05 N HCl
Lactobacilli Agar AOAC
Folic Acid
Incubator (35-37°C)
Micro Inoculum Broth
Centrifuge
Spectrophotometer

Method of Preparation

Folic Acid Casei Medium

1. Suspend 9.4 grams in 100 ml distilled or deionized water.
2. Add 50 mg ascorbic acid if standard and test samples are not prepared in Folic Buffer A.
3. Boil for 1-2 minutes.
4. Dispense 5 ml amounts into tubes, evenly dispersing any precipitate.
5. Add standard or test samples.
6. Adjust tube volume to 10 ml with distilled or deionized water.
7. Autoclave at 121°C for 5 minutes.

Folic Buffer A Dried

1. Dissolve the contents of one vial (15.4 grams) in 1 liter distilled or deionized water.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Preparation of Stock Cultures and Inoculum

Prepare stock cultures of the test organism, *L. casei* subsp. *rhamnosus* ATCC® 7469, by stab inoculation into prepared tubes of Lactobacilli Agar AOAC. Incubate the cultures at 35-37°C for 18-24 hours. Store cultures in the refrigerator at 2-8°C. Stock transfers are made at monthly intervals.

Prepare the inoculum for assay by subculturing from a stock culture of *L. casei* subsp. *rhamnosus* into a tube containing 10 ml prepared Micro Inoculum Broth. Incubate at 35-37°C for 16-18 hours. Under aseptic conditions, centrifuge the tubes to sediment the cells and decant the supernatant. Wash the cells in 10 ml sterile single-strength Folic Acid Casei Medium. Resediment the cells by centrifuging aseptically and decant the supernatant. Repeat washing two more times. After the third washing, resuspend the cells in 10 ml sterile single-strength medium and dilute 1 ml with 99 ml of the same medium. One drop of this suspension is used to inoculate each of the assay tubes. Read the growth response of the assay tubes turbidimetrically after 18-24 hours incubation at 35-37°C. (Some laboratories use 0.85% saline instead of the single-strength basal medium to wash and dilute the inoculum.)

Preparation of the Standard

It is essential that a standard curve be constructed for each separate assay. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated. The standard curve may be obtained by using folic acid at levels of 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ng per assay tube (10 ml).

The folic acid required for preparation of the standard curve may be prepared as follows:

Dissolve 50 mg dried folic acid in about 30 ml 0.01 N NaOH and 300 ml distilled water. Adjust to pH 7-8 with 0.05 N HCl and dilute to 500 ml with distilled water. Dilute 10 ml of this solution with 500 ml distilled water. Further dilute 1 ml in 1 liter distilled water to make a stock solution containing 2 ng per ml folic acid. Prepare the standard solution containing 0.2 ng per ml folic acid by diluting 10 ml of stock solution with 90 ml of Folic Buffer A, Dried solution. Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml per assay tube.

Prepare the stock solution fresh daily.

Preservation of Serum Specimens

1. Allow the blood specimen to clot and the serum to separate from the clot.
2. Aspirate the serum into a clean dry tube and centrifuge to remove any cells that may be present. Avoid hemolysis. Dispense 5 ml of each serum sample into clean dry test tubes and add 25 mg ascorbic acid to each tube.
3. If the test is not begun immediately, place tubes in a freezer and hold below -20°C.

Preparation of Serum Specimen

1. Thaw the serum containing ascorbic acid.
2. Add 5 ml of the uniform sample to 45 ml rehydrated Folic Buffer A, Dried.
3. Incubate the serum-buffer solution at 37°C for 90 minutes. Autoclave the incubated mixture at 121°C for 2.5 minutes.
4. Remove the coagulated protein by centrifuging and transfer the clear supernatant to a clean dry tube. The clear solution is the sample to use in the folic acid assay.

Procedure for Total Folic Acid

1. Use 0.5, 1.0, 1.5 ml or other volumes of the prepared serum extracts as described above.
2. Fill each assay tube with 5 ml of rehydrated Folic Acid Casei Medium and sufficient distilled or deionized water to give a total volume of 10 ml per tube.
3. Autoclave tubes at 121°C for 5 minutes.
4. Add 1 drop of inoculum described under **Preparation of Stock Culture and Inoculum** to each assay.
5. Incubate at 35-37°C for 18-24 hours. Tubes are refrigerated for 15-30 minutes to stop growth before reading turbidimetrically.

Results

The amount of folic acid in the test samples can be determined by interpolating the results with the values obtained on the standard curve, taking into consideration the dilutions of the samples.

Limitations of the Procedure

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

References

1. **Flynn, Williams, O'Dell, and Hogan.** 1951. Anal. Chem. **23**:180.
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Packaging

Folic Acid Casei Medium	100 g	0822-15
Folic Buffer A, Dried	6 x 15.4 g	3246-33

Fraser Broth

Bacto® Fraser Broth Base · Fraser Broth Supplement

Intended Use

Bacto Fraser Broth Base is used with Bacto Fraser Broth Supplement in selectively enriching and detecting *Listeria*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985,[3] and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has indicated that the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté, and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, silage and river water.[6]

Bacto Fraser Broth Base and Bacto Fraser Broth Supplement are based on the formulation of Fraser and Sperber.[7] The medium is use in the rapid detection of *Listeria* from food[8] and environmental samples. Many common food contaminants such as streptococci, enterococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* interfere with the isolation of *Listeria monocytogenes*.[9]

Listeria species grow over a pH range of 5.0-9.6, and survive in food products with pH levels outside these parameters.[10] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Principles of the Procedure

Bacto Tryptose, Bacto Beef Extract and Bacto Yeast Extract provide nitrogen, vitamins and minerals. Sodium phosphate and potassium phosphate are buffering agents. Differentiation is aided by including ferric ammonium citrate in the final medium. Since all *Listeria* species hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the medium by cultures containing esculin-hydrolyzing bacteria is the result of the formation of 6,7-dihydroxycoumarin that reacts with the ferric ions.[7]

Selectivity is provided by the presence of lithium chloride, nalidixic acid and acriflavine in the formula. The high salt tolerance of *Listeria* is used as a means to inhibit growth of enterococci.

Formula

Fraser Broth Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Sodium Chloride	20 g
Sodium Phosphate, Dibasic	9.6 g
Potassium Phosphate, Monobasic	1.35 g
Esculin	1 g
Nalidixic Acid	0.02 g
Acriflavine HCl	0.024 g
Lithium Chloride	3 g

Final pH 7.2 ± 0.2 at 25°C

Fraser Broth Supplement

Ingredients per 10 ml vial

Ferric Ammonium Citrate	0.5 g

Precautions

1. For Laboratory Use.
2. **Fraser Broth Base**:

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Fraser Broth Supplement:

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Bacto Fraser Broth Supplement at 2-8°C.

Store the prepared medium at 2-8°C.

User Quality Control

Identity Specifications

Fraser Broth Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, clear to slightly opalescent with a fine precipitate.
Prepared Tubes:	Medium amber, clear to slightly opalescent with a fine precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.2 ± 0.2

Bacto Fraser Broth Supplement

Solution Appearance:	Dark brown solution.

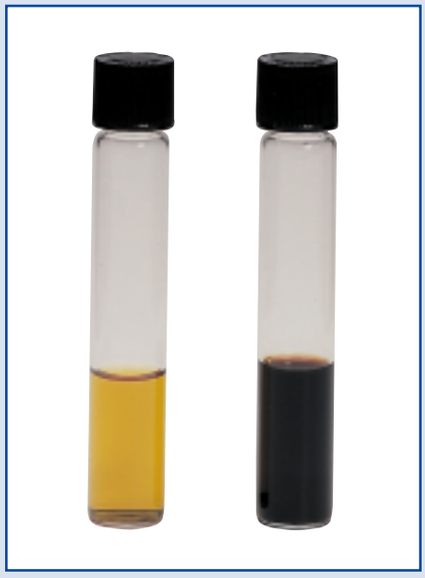
Cultural Response

Prepare Fraser Broth Base per label directions. Add Fraser Broth Supplement. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ESCULINE REACTION
Escherichia faecalis	29212*	1,000-2,000	marked to complete inhibition	–
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	–
Listeria monocytogenes	19114	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube — *Listeria monocytogenes* ATCC® 19114

Expiration Date

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

Procedure

Materials Provided

Fraser Broth Base
Fraser Broth Supplement

Materials Required But Not Provided

Flasks with closure
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Test tubes with closures
Incubator (30°C)
Incubator (35°C)

Method of Preparation

1. Suspend 55 grams of Fraser Broth Base in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Aseptically add 10 ml Fraser Broth Supplement. Mix well.
5. Dispense into tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

To isolate *Listeria monocytogenes* from processed meats and poultry, the following procedure is recommended by the U.S.D.A.[8]

1. Add 25 grams of test material to 225 ml of UVM Modified Listeria Enrichment Broth and mix or blend thoroughly.
2. Incubate for 20-24 hours at 30°C.
3. Transfer 0.1 ml of the incubated broth to Fraser Broth. Incubate at 35°C for 26 ± 2 hours.
4. At 24 and 48 hours, streak the Fraser Broth culture to Modified Oxford Agar.
5. Incubate the Modified Oxford plates at 35°C for 24-48 hours.

Results

1. Examine agar plates for suspect colonies. For further identification and confirmation of *Listeria* spp., consult appropriate references.[8,10,11,12]
2. Rapid slide and macroscopic tube tests can be used for definitive serological identification.

Limitations of the Procedure

1. Since *Listeria* species other than *L. monocytogenes* can grow on these media, an identification of *Listeria monocytogenes* must be confirmed by biochemical and serological testing.[11,12]
2. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

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* (n. sp.). J. Path. Bact. **29:**407- 439.
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12. **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.

Packaging

Fraser Broth Base	500 g	0219-17
	2 kg	0219-07
Fraser Broth Supplement	6 x 10 ml	0211-60*

*Store at 2-8°C

Bacto® GC Medium Base · Bacto Supplement B Bacto Supplement VX · Bacto Hemoglobin Bacto Antimicrobic Vial CNV · Bacto Antimicrobic Vial CNVT

Intended Use

Bacto GC Medium Base is used with various additives in isolating and cultivating *Neisseria gonorrhoeae* and other fastidious microorganisms.

Bacto Supplement B with Bacto Reconstituting Fluid B is used for supplementing media to culture fastidious organisms, particularly *Neisseria gonorrhoeae* and *Haemophilus influenzae*.

User Quality Control

Identity Specifications

GC Medium Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water upon boiling; light to medium amber, opalescent, may have slight precipitate, "ground glass" appearance.
Prepared Medium:	With Hemoglobin and Supplement: chocolate brown, opaque.
Reaction of 3.6% Solution at 25°C:	pH 7.2 ± 0.2

Hemoglobin

Dehydrated Appearance:	Dark brown, fine, free-flowing.
Solution:	2% solution, insoluble in distilled or deionized water; chocolate brown, opaque with a dispersed precipitate.
Reaction of 2% Solution at 25°C:	pH 8.2 ± 0.2

Supplement B

Lyophilized Appearance:	Tan to reddish brown lyophilized powder or cake.
Rehydrated Appearance:	Medium to dark amber may have a reddish tint, clear to slightly opalescent solution.
Reconstituting Fluid:	Colorless, clear solution.
Sterility Test:	Satisfactory.
Reaction of Solution at 25°C:	pH 6.5-7.2

Supplement VX

Lyophilized Appearance:	Pink, lyophilized powder.
Rehydrated Appearance:	Pink, clear solution without precipitate.
Reconstituting Fluid:	Colorless, clear solution.
Sterility Test:	Satisfactory.
Reaction of Solution at 25°C:	pH 0.75-2.5

continued on following page

Bacto Supplement VX with Bacto Reconstituting Fluid VX is used to culture fastidious microorganisms, particularly *Neisseria gonorrhoeae*.

Bacto Hemoglobin is used in preparing microbiological culture media.

Bacto Antimicrobic Vial CNV and Bacto Antimicrobic Vial CNVT are sterile lyophilized preparations containing inhibitory agents to be used in selective media for culturing *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

Also Known As

GC Medium is also referred to as Chocolate Agar Base, Chocolate Agar, Enriched and GC Agar.

Summary and Explanation

In 1945, Johnston[1] described a medium that could successfully produce colonies of *N. gonorrhoeae* in 24 rather than 48 hours. The accelerated growth rates were primarily due to the decreased agar content (solidity) of the media. GC Medium Base was introduced in 1947 with reduced agar content. While investigating the growth rate of some gonococcal strains, a medium containing the growth factors glutamine and cocarboxylase, was found to improve recovery.[2,3] From this discovery, Supplement B was developed. In a comparative study[4] of 12 different media, an enriched Chocolate Agar prepared with GC Medium Base, Hemoglobin and Supplement B proved superior for isolating *N. gonorrhoeae.*

Supplement B w/ Reconstituting Fluid is a sterile yeast concentrate for use in supplementing media for microorganisms with exacting growth requirements. It is recommended for use in the preparation of chocolate agar described by Christensen and Schoenlein.[5]

Supplement VX w/ Reconstituting Fluid is a sterile lyophilized concentrate. Supplement B and Supplement VX are recommended for enriching GC Medium Base, Proteose No. 3 Agar, Thayer-Martin Medium and Modified Thayer-Martin Medium.

Hemoglobin, an autoclavable preparation of beef blood, is prepared according to described by Spray.[6] Hemoglobin provides hemin, which is required by *Haemophilus* species and enhances growth of *Neisseria* species.

In 1964, Thayer and Martin[7] formulated a selective medium incorporating the antibiotics polymyxin B and ristocetin into GC Agar with added hemoglobin and yeast supplement B. Thayer and Martin[8] improved their medium by replacing the two original antibiotics with a new microbial solution of colistin, vancomycin and nystatin (CVN). In 1970, Martin and Lester[9] improved the new Thayer-Martin (TM) medium by increasing the agar and glucose content and by incorporating an additional antibiotic, trimethoprim lactate (T) into the formulation. This improved medium is called Modified Thayer-Martin (MTM) Medium. Antimicrobic Vial CNV and Antimicrobic Vial CNVT are used in the preparation of Thayer-Martin (TM) Medium and Modified Thayer-Martin, respectively.

Martin and Lewis[10] further improved selectivity of MTM by increasing the concentration of vancomycin and replacing nystatin with anisomycin for greater inhibition of yeasts; this is known as Martin-Lewis (ML) Agar Medium. Transgrow Medium is a transport medium system incorporating either MTM or ML formulations.[11]

Principles of the Procedure

GC Medium Base is employed as the basal medium in the preparation of Chocolate Agar Enriched, Thayer-Martin Medium and Modified Thayer-Martin Medium.

Proteose Peptone No. 3 provides nitrogen, vitamins and amino acids in GC Medium Base. Corn Starch absorbs any toxic metabolites that are produced, Potassium Phosphate, Dibasic and Monobasic buffer the medium. Sodium Chloride maintains osmotic balance. Bacto Agar is a solidifying agent.

Chocolate Agar is prepared from GC Medium Base with the addition of 2% Hemoglobin. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* and enhanced growth of *Neisseria*.

The growth rate of *Neisseria* and *Haemophilus* is improved with the addition of 1% Supplement B or VX, providing the growth factors glutamine and cocarboxylase. Supplement B contains yeast concentrate, glutamine, coenzyme, cocarboxylase, hematin and growth factors. Supplement VX is a sterile, defined lyophilized concentrate of essential growth factors. Supplement VX supplies vitamins, amino acids, coenzymes, dextrose and other factors to improve the growth of *Haemophilus* and *Neisseria* species.

Antimicrobic Vial CNV and Antimicrobic Vial CNVT are antimicrobial agents used as inhibitors in the selective media, Thayer-Martin Medium and Modified Thayer-Martin Medium.

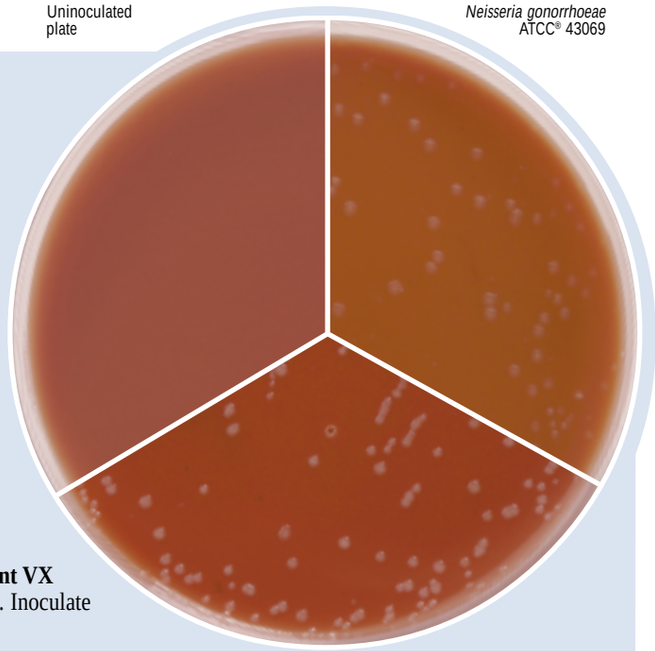
Formula

GC Medium Base

Formula Per Liter

Bacto Proteose Peptone No. 3	15 g
Corn Starch	1 g
Potassium Phosphate, Dibasic	4 g
Potassium Phosphate, Monobasic	1 g
Sodium Chloride	5 g
Bacto Agar	10 g

Final pH 7.2 ± 0.2 at 25°C



User Quality Control cont.

Antimicrobic Vial CNV

Lyophilized Appearance:	Pale yellow, dry cake or powder.
Rehydrated Appearance:	Off-white to pale yellow, opalescent to opaque even suspension.
Solubility:	Not completely soluble in distilled water, but must be evenly suspendable.
Microbial Limits Test:	Negative.

Antimicrobic Vial CNVT

Lyophilized Appearance:	Pale yellow, dry cake or powder.
Rehydrated Appearance:	Off-white to pale yellow, opalescent to opaque even suspension.
Solubility:	Not completely soluble in distilled water, but must be evenly suspendable.
Microbial Limits Test:	Negative.

Cultural Response

GC Medium Base, Hemoglobin 2%, Supplement B or Supplement VX

Prepare Chocolate Agar with GC Medium Base, per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Haemophilus influenzae	10211	30-300	good
Neisseria gonorrhoeae	43069	30-300	good

GC Medium Base, Hemoglobin 2%, Supplement B or Supplement VX, Antimicrobic Vial CNV or CNVT

Prepare Thayer-Martin Medium or Modified Thayer-Martin Medium with GC Medium Base per label directions, enriched with Antimicrobic Vial CNV or Antimicrobic Vial CNVT. Inoculate and incubate at 35 ± 2°C under CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Candida albicans	60193	1,000	partial inhibition
Escherichia coli	25922*	1,000	marked to complete inhibition
Neisseria gonorrhoeae	43069	100-1,000	good
Neisseria meningitidis	13090*	100-1,000	good
Neisseria sicca	9913*	100-1,000	marked to complete inhibition
Staphylococcus epidermidis	12228*	1,000	marked to complete inhibition

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disk Technical Information.

Hemoglobin

An autoclavable preparation of beef blood prepared according to the procedure described by Spray.[6]

Supplement B

Processed to preserve both the thermolabile and thermostable growth accessory factors of fresh yeast, contains glutamine, coenzyme (V factor), cocarboxylase and other growth factors, as well as hematin (X factor).

Supplement VX

Ingredients per 10 ml Vial

Adenine Sulfate	10 mg
p-Aminobenzoic Acid	0.25 mg
Cocarboxylase	2 mg
L-Cysteine HCl	259 mg
L-Cystine	11 mg
Diphosphopyridine Nucleotide	3.5 mg
Ferric citrate	0.3 mg
L-Glutamine	200 mg
Guanine HCl	0.3 mg
Thiamine HCl	0.06 mg
Vitamin B_{12} (Cyanocobalamin)	0.2 mg
Bacto Dextrose	1 mg

Antimicrobial Vial CNV

A sterile, lyophilized preparation containing 7,500 µg Colistin Sulfate, 12,500 units Nystatin and 3,000 µg Vancomycin per 10 ml.

Antimicrobial Vial CNVT

A sterile lyophilized preparation containing 7,500 µg Colistin Sulfate, 12,500 units Nystatin, 3,000 µg Vancomycin and 5,000 µg Trimethoprim per 10 ml.

Precautions

1. For Laboratory Use.
2. **Antimicrobic Vial CNV**

   HARMFUL. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Ears, Lungs, Thorax.

   Antimicrobic Vial CNVT

   HARMFUL. HARMFUL BY INHALATION AND IF SWALLOWED. (US) MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Thorax.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store GC Medium Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Hemoglobin below 30°C. Store Hemoglobin 2% at 15-30°C.

Store Supplements B and VX at 2-8°C.

Store Antimicrobic Vials CNV and CNVT at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

GC Medium Base
Hemoglobin, Hemoglobin 2%
Supplement B or VX
Antimicrobial Vial CNV or CNVT

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C).
Sterile Petri dishes or tubes

Method of Preparation

Supplement B
Supplement VX

1. Aseptically rehydrate Supplement B and Supplement VX with 10 ml or 100 ml of the corresponding Reconstituting Fluid, as appropriate.
2. Rotate the vial to dissolve completely.

Hemoglobin

1. Place 10 grams of Hemoglobin in a dry beaker.
2. Measure 500 ml distilled or deionized water.
3. Add approximately 100 ml amounts to the Hemoglobin, stirring well after each addition. Use a spatula to break up clumps.
4. Transfer to flasks as desired for autoclaving.
5. Autoclave at 121°C for 15 minutes.
6. Cool to 45-50°C.
7. Swirl flask to reestablish complete solution and add to an equal amount of double-strength sterile agar base cooled to 45-50°C.

Hemoglobin 2%

1. Shake the bottle to resuspend any sedimented hemoglobin before use.

Antimicrobial Vial CNV
Antimicrobial CNVT

1. Aseptically rehydrate Antimicrobial Vial CNV or Antimicrobial CNVT with the appropriate amount of sterile distilled or deionized water, as indicated on the product label.
2. Rotate the vial to dissolve completely.

Chocolate Agar, Enriched

1. Suspend 7.2 grams GC Medium Base in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 ml Hemoglobin Solution 2%.
5. Aseptically add 2 ml Supplement B or Supplement VX. Mix well.
6. Dispense into sterile Petri dishes or tubes as desired.

Thayer-Martin Medium

1. Suspend 7.2 grams GC Medium Base in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 ml Hemoglobin Solution 2%.
5. Aseptically add 2 ml Supplement B or Supplement VX.
6. Aseptically add 2 ml rehydrated Antimicrobial Vial CNV to the medium.
7. Dispense into sterile Petri dishes.

Modified Thayer-Martin Medium

1. Suspend 7.2 grams GC Medium Base in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 ml Hemoglobin Solution 2% and 0.3 grams dextrose to the medium.
5. Aseptically add 2 ml Supplement B or Supplement VX.
6. Aseptically add 2 ml of rehydrated Antimicrobial Vial CNVT to the medium.
7. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of *Neisseria* and *Haemophilus*, consult the procedures outlined in the references.[12,13,14]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. GC Medium Base is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
3. Improper specimen collection, environment, temperature, CO_2 level, moisture and pH can adversely affect the growth and viability of the organism.
4. Inactivation or deterioration of antibiotics in Thayer-Martin or Modified Thayer- Martin may allow growth of contaminants.
5. GC Medium Base has sufficient buffering capacity to offset the very low pH of the small amount of Supplement VX added. The pH of some media has to be adjusted with 1% NaOH after the addition of Supplement VX.

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12. **Isenberg, H. D. (ed.)**. 1992. Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, D.C.
13. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
14. **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.

Packaging

GC Medium Base	100 g	0289-15
	500 g	0289-17
	2 kg	0289-07
	10 kg	0289-08
Hemoglobin	100 g	0136-15
	500 g	0136-17
	2 kg	0136-07
	10 kg	0136-08
Hemoglobin 2% Solution	6 x 100 ml	3248-73
Supplement B w/Reconstituting Fluid	6 x 100 ml	0276-60
	100 ml	0276-72
Supplement VX w/Reconstituting Fluid	6 x 10 ml	3354-60
	100 ml	3354-72
Antimicrobic Vial CNV	6 x 10 ml	3260-60
Antimicrobic Vial CNVT	6 x 10 ml	3198-60
	100 ml	3198-72

Bacto® GN Broth, Hajna

Intended Use

Bacto GN Broth, Hajna is used for isolating and cultivating gram-negative microorganisms.

Also Known As

Gram Negative (GN) Broth[1]

Hajna GN Broth[1]

Gram Negative Enrichment Broth[1]

Summary and Explanation

Hajna[2,3] formulated Gram Negative (GN) Broth as an enrichment medium for enteric gram-negative bacilli, especially *Salmonella* and *Shigella*, from clinical and non-clinical specimens. Croft and Miller[4] demonstrated improved recovery of *Shigella* using GN Broth enrichment compared to direct inoculation of agar media. Taylor and Schelhart[5] reported improved recovery of both *Salmonella* and *Shigella* when using GN Broth enrichment compared to direct inoculation of agar media. Taylor and Schelhart[6] showed GN Broth to be superior to selenite enrichment medium for recovering *Shigella*.

GN Broth, Hajna is recommended as an enteric enrichment broth for clinical specimens[7,8] and as a nonselective enrichment broth for foods[9] to recover *Salmonella* and *Shigella*.

Principles of the Procedure

GN Broth, Hajna contains Tryptose as a source of carbon, nitrogen, vitamins and minerals. Dextrose and D-Mannitol are carbohydrates. Sodium Citrate and Sodium Desoxycholate inhibit growth of gram-positive bacteria and of coliforms other than *Salmonella* and *Shigella*. Dipotassium Phosphate and Monopotassium Phosphate buffer the medium.

The higher concentration of mannitol over dextrose favors growth of mannitol-fermenting *Salmonella* and *Shigella* over mannitol non-fermenting species, such as *Proteus*.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white to light tan, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.
Prepared Medium:	Light amber, clear to very slightly opalescent.
Reaction of 3.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Salmonella typhimurium	14028*	100-1,000	good
Shigella flexneri	12022*	100-1,000	good
Enterococcus faecalis	19433*	1,000-2,000	none to poor

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

GN Broth, Hajna

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	1 g
Bacto D-Mannitol	2 g
Sodium Citrate	5 g
Sodium Desoxycholate	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

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

GN Broth, Hajna

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve 39 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Avoid overheating.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Growth of gram-negative organisms, especially *Salmonella* and *Shigella* species, is enhanced.

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, et al. (ed.), 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 (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

GN Broth, Hajna	500 g	0486-17

Bacto® Gelatin
Bacto Gelatone

Intended Use

Bacto Gelatin is used in preparing microbiological culture media.

Bacto Gelatone is used in preparing microbiological culture media.

Also Known As

Gelatone is also referred to as Gelatin Peptone.

Summary and Explanation

Gelatin is a protein of uniform molecular constitution derived chiefly by the hydrolysis of collagen.[1] Collagens are a class of albuminoids found abundantly in bones, skin, tendon, cartilage and similar animal tissues.[1]

Koch[1] introduced gelatin into bacteriology when he invented the gelatin tube method in 1875 and the plate method in 1881. This innovation, a solid culture method, became the foundation for investigation of the propagation of bacteria.[1] However, gelatin-based media were soon replaced by media containing agar as the solidifying agent.

Gelatin is used in culture media for determining gelatinolysis (elaboration of gelatinases) by bacteria. Levine and Carpenter[2] and Levine and Shaw[3] employed gelatin media in their studies of gelatin liquefaction. Garner and Tillett[4] used culture media prepared with gelatin to study the fibrinolytic activity of hemolytic streptococci.

Gelatin is a high grade gelatin in granular form which may be used as a solidifying agent or may be incorporated into culture media for various uses. Gelatin is used in Nutrient Gelatin, Motility GI Medium, Motility Medium S, Stock Culture Agar and Dextrose Starch Agar. Media containing gelatin are specified in Standard Methods[5,6] for multiple applications.

Gelatone, a granular pancreatic digest of gelatin, is deficient in carbohydrates. It is distinguished by low cystine and tryptophan content. Gelatone is used as an ingredient in media for fermentation studies and, by itself, to support growth of non-fastidious microorganisms.

Principles of the Procedure

The melting point of a 12% concentration of Gelatin is between 28 and 30%C, which allows it to be used as a solidifying agent. Certain microorganisms elaborate gelatinolytic enzymes (gelatinases) which hydrolyze gelatin, causing liquefaction of a solidified medium or preventing the gelation of a medium containing gelatin. Gelatin is also used as a source of nitrogen and amino acids.

Gelatone is a peptone from gelatin obtained by digesting gelatin with pancreatin.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated product below 30°C. The dehydrated product is very hygroscopic. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Gelatin

Gelatone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Preparation varies depending on the medium being prepared.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Gelatin or Gelatone.

Results

Refer to appropriate references and procedures for results.

References

1. **Gershenfeld, L., and L. F. Tice.** 1941. Gelatin for bacteriological use. J. Bacteriol. **41**:645-652.
2. **Levine and Carpenter.** 1923. J. Bacteriol. **8**:297.
3. **Levine and Shaw.** 1924. J. Bacteriol. **9**:225.
4. **Garner and Tillett.** 1934. J. Exp. Med. **60**:255.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Gelatin	100 g	0143-15
	500 g	0143-17
	10 kg	0143-08
Gelatone	500 g	0657-17

User Quality Control

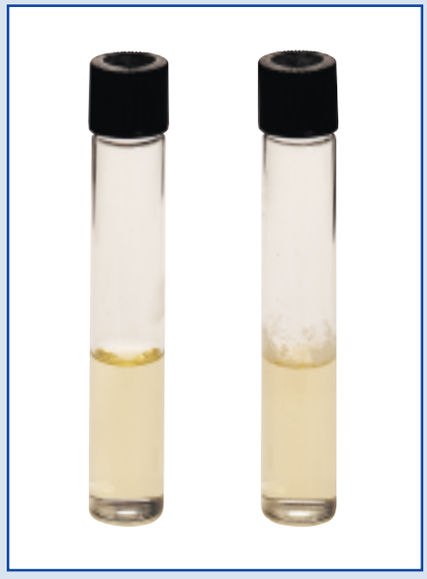
Identity Specifications

Gelatin

Dehydrated Appearance:	Light beige, free-flowing, homogeneous granules.
Solution:	12% solution, soluble in distilled or deionized water on slight heating in a 50-55°C waterbath. Solution is light amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Gel:	Very light amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 12% Solution at 25°C:	pH 6.8 ± 0.2

Gelatone

Dehydrated Appearance:	Tan, free-flowing granules.
Solution:	10% solution, soluble in distilled or deionized water: 1%-Very light to light amber, clear; 2%-Light to medium amber, clear; 10%-Medium to dark amber, clear to very slightly opalescent.
Reaction of 2% Solution at 25°C:	pH 6.3-7.6



Uninoculated tube — *Bacillus subtilis* ATCC® 6633

Cultural Response

Gelatin

Prepare a 12% Gelatin solution in 0.8% Nutrient Broth and sterilize. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 18-48 hours or for up to two weeks for the gelatinase test. To read gelatinase, refrigerate until well chilled and compare to uninoculated tubes. Tubes positive for gelatinase will remain liquid.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	GELATINASE
Escherichia coli	25922*	100-1,000	good	–
Clostridium sporogenes	11437	100-1,000	good	+
Bacillus subtilis†	6633	100-1,000	good	+

†*Bacillus subtilis* is available as Bacto Subtilis Spore Suspension.

Gelatone

Prepare a 2% Gelatone solution in 0.5% saline; adjust pH to 7.2-7.4; add 1.5% Bacto Agar, boil and sterilize. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella suis	4314	100-1,000	good growth
Escherichia coli	25922*	100-1,000	good growth
Staphylococcus aureus	25923*	100-1,000	good growth

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Bacto® Giolitti-Cantoni Broth Base
Bacto Potassium Tellurite Solution 3.5%

Intended Use

Bacto Giolitti-Cantoni Broth Base is used with Bacto Potassium Tellurite Solution 3.5% in enriching *Staphylococcus aureus* from foods during isolation procedures.

Summary and Explanation

Giolitti and Cantoni[1] described a broth medium with added potassium tellurite and a test procedure for enriching small numbers of staphylococci in foods. Mossel et al[2] recommended Giolitti-Cantoni Broth for detecting *Staphylococcus aureus* in dried milk and other infant foods where the organism should be absent from 1 g of test material.

The International Dairy Federation (IDF) and American Public Health Association recommend a procedure for detecting *S. aureus* in dairy products using Giolitti-Cantoni Broth as an enrichment medium from which selective media are inoculated.[3, 4]

Principles of the Procedure

Giolitti-Cantoni Broth Base contains Tryptone and Beef Extract as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. D-Mannitol is the carbohydrate source. Sodium Pyruvate stimulates growth of staphylococci. Lithium Chloride inhibits gram-negative bacilli. Potassium Tellurite Solution 3.5% supplies potassium tellurite, which in combination with glycine, inhibits gram-positive bacteria other than staphylococci.

Formula

Giolitti-Cantoni Broth Base

Formula Per Liter

Bacto Tryptone	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Bacto D-Mannitol	20 g
Sodium Chloride	5 g
Lithium Chloride	5 g
Glycine	1.2 g
Sodium Pyruvate	3 g

Final pH 6.9 ± 0.2 at 25°C

Potassium Tellurite Solution 3.5%

A filter-sterilized solution of potassium tellurite in distilled water.

Precautions

1. For Laboratory Use.

User Quality Control

Identity Specifications

Giolitti-Cantoni Broth Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.42% solution, soluble in distilled or deionized water on warming. Solution is medium amber, clear without significant precipitate.
Prepared Medium:	Medium amber, clear without significant precipitate.
Reaction of 5.42% Solution at 25°C:	pH 6.9 ± 0.2

Potassium Tellurite Solution 3.5%

Appearance:	Colorless, clear solution, may have a fine precipitate.

Cultural Response

Prepare Giolitti-Cantoni Broth per label directions. Inoculate per Test Procedure and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	1,000-2,000	inhibited	no blackening
Micrococcus luteus	10240	1,000-2,000	inhibited	no blackening
Staphylococcus aureus	6538	100-1000	good	blackening
Staphylococcus aureus	25923*	100-1000	good	blackening

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Staphylococcus aureus* ATCC® 6538

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

2. **Giolitti-Cantoni Broth Base**

   HARMFUL. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGANS: Blood, Kidneys, Nerves.

   Potassium Tellurite Solution

   WARNING! HARMFUL IF SWALLOWED. CAUSES IRRITATION.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store dehydrated Giolitti-Cantoni Broth Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Potassium Tellurite Solution 3.5% at 15-30°C

Expiration Date

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

Procedure

Materials Provided

Giolitti-Cantoni Broth Base
Potassium Tellurite Solution 3.5%

Materials Required but not Provided

Glassware
Tubes 20 X 200 mm
Distilled or deionized water
Autoclave
Incubator (35°C)
Sterile paraffin wax or sterile mineral oil

Method of Preparation

1. Suspend 54.2 grams Giolitti-Cantoni Broth Base in 1 liter distilled or deionized water.
2. Warm gently to dissolve completely.
3. Dispense 19 ml amounts into 20 x 200 mm tubes.
4. Autoclave at 121°C for 15 minutes. Cool to 15-30°C.
5. Aseptically add 0.3 ml Potassium Tellurite Solution 3.5% per 19 ml tube or 0.03 ml when testing meat products or quality control organisms.
6. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate 1 gram or 1 ml of test sample (0.1 gram or 0.1 ml when testing meat or meat products) and 1 ml aliquots of each of a suitable decimal dilution series of the test sample into duplicate tubes.
2. Overlay each tube with 5 ml sterile molten paraffin wax to an approximate height of 2 cm.
3. Incubate at 35 ± 2°C 40-48 hours.
4. Examine daily.

Results

Read tubes for blackening of the medium (a positive reaction) or no blackening (a negative reaction). If blackening occurs, subculture to Baird Parker Agar to confirm the isolation of *S. aureus*.

References

1. **Giolitti, G., and C. Cantoni**. 1966. A medium for the isolation of staphylococci from foodstuffs. J. Appl. Bacteriol. **29**:395-398.
2. **Mossel, D. A. A., G. A. Harrewijn, and J. M. Elzebroek**. 1973. UNICEF.
3. **International Dairy Federation**. 1978. IDF Standard 60A:1978. International Dairy Federation.
4. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Giolitti-Cantoni Broth Base	500 g	1809-17
Potassium Tellurite Solution 3.5%	25 ml	1814-65

Bacto® HC Agar Base

Intended Use

Bacto HC Agar Base, when supplemented with Polysorbate 80, is used for enumerating molds in cosmetic products.

Summary and Explanation

Methods for isolating molds from cosmetic products require incubation for 5 to 7 days using traditional agar media.[1] In 1986, Mead and O'Neill[2] described a new medium, HC Agar, for enumerating molds in cosmetic products that decreased incubation time to 3 days at 27.5 ± 0.5°C. HC Agar Base, based on the HC Agar formula of Mead and O'Neill, is supplemented with Polysorbate 80 to prepare HC Agar.

Principles of the Procedure

HC Agar Base contains Tryptone and Proteose Peptone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose provides a source of fermentable carbohydrate. Ammonium Chloride and Magnesium Sulfate provide essential ions. Disodium and Monopotassium Phosphates