

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'Neill² 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

buffer the pH to near neutrality. Sodium Carbonate inactivates low levels of preservatives that are active at a more acidic pH (e.g., benzoic acid). Chloramphenicol inhibits bacteria, including *Pseudomonas aeruginosa* and *Serratia marcescens*, that are potential contaminants of cosmetic products. Polysorbate 80 neutralizes preservatives and sequesters surfactants that may be present in residual amounts from the product sample.² Bacto Agar is the solidifying agent.

Formula

HC Agar Base

Formula Per Liter	
Bacto Tryptone	2.5 g
Bacto Proteose Peptone	2.5 g
Bacto Yeast Extract	5 g
Bacto Dextrose	20 g
Disodium Phosphate	3.5 g
Monopotassium Phosphate	3.4 g
Ammonium Chloride	1.4 g
Magnesium Sulfate	0.06 g
Chloramphenicol	0.1 g
Sodium Carbonate	1 g
Bacto Agar	15 g
Final pH 7.0 ± 0.2 at 25°C	

Precautions

1. For Laboratory Use.
2. **TOXIC. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE CANCER. POSSIBLE RISK OF**

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light to light beige, free-flowing, homogeneous.
Solution:	5.45% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, slightly opalescent to opalescent, may have a slight precipitate.
Prepared Medium:	Medium amber with yellow tint, very slightly to slightly opalescent, no significant precipitate.
Reaction of 5.45% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare HC Agar Base per label directions. Supplement with Polysorbate 80. Inoculate and incubate the plates at 27.5 ± 0.5°C for 65-72 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Aspergillus niger</i>	16404	100-1000	good
<i>Pseudomonas aeruginosa</i>	10145	1,000-2,000	none to poor
<i>Serratia marcescens</i>	13880	1,000-2,000	none to poor

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

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, Eye/Ear, Muscles, Nerves, Urogenital.

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

HC Agar Base

Materials Required but not Provided

Polysorbate 80
Glassware
Sterile Petri dishes
Autoclave
Incubator (27.5 ± 0.5°C)

Method of Preparation

1. Suspend 54.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 20 ml Polysorbate 80.
4. Autoclave at 121°C for 15 minutes.
5. Dispense into petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines.¹

Test Procedure

1. Process each specimen as appropriate for that specimen and inoculate directly onto the surface of the medium.¹ Inoculate duplicate plates.
2. Incubate plates aerobically at 27.5 ± 0.5°C.
3. Examine plates for growth and recovery after 72 hours incubation.
4. Count mold colonies from duplicate plates and record average count as mold count per gram or milliliter of sample.

Results

Mold cultures should yield good growth and recovery. Bacteria should be inhibited.

Limitations of the Procedure

1. The $27.5 \pm 0.5^\circ\text{C}$ incubation temperature is critical for obtaining statistically significant mold counts after three days using this medium.
2. Nutritional requirements of organisms vary. Some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1995. Microbiological methods in cosmetics, p. 23-01-23.12. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
2. **Mead, C., and J. O'Neill.** 1986. A three-day mold assay for cosmetics and toiletries. *J. Soc. Cosmet. Chem.* **37**:49-57.

Packaging

HC Agar Base 500 g 0685-17

Bacto® m HPC Agar

Intended Use

Bacto m HPC Agar is used for enumerating heterotrophic organisms in treated potable water and other water samples with low counts by membrane filtration.

Also Known As

m HPC Agar is also known as m-Heterotrophic Plate Count Agar and previously as membrane filter Standard Plate Count Agar, m-SPC Agar.

Summary and Explanation

m HPC Agar was developed by Taylor and Geldreich in 1979 in their pursuit of a suitable Standard Methods medium to use with the membrane filter procedure.¹ m HPC Agar was evaluated by many investigators who reported it as a suitable alternate medium for standard plate counts.^{2,3,4} This medium is recommended for the membrane filter method in the 19th edition of *Standard Methods for the Examination of Water and Wastewater*.⁵

The advantages of the membrane filter procedure over the standard plate count method have been described by many investigators.^{6,7,8}

The volume of inoculum is limited with both pour and spread plate techniques while the membrane filter method enables the use of large samples, which is desirable for water with low counts.

Principles of the Procedure

In m HPC Agar, Bacto Peptone provides sufficient nitrogen and carbon as well as other nutrients. Gelatin at 2.5% concentration eliminates problems of liquefaction and spreading colonies. Bacto Agar is a solidifying agent.

Formula**m HPC Agar**

Formula Per Liter

Bacto Peptone	20 g
Bacto Gelatin	25 g
Bacto Agar	15 g
Final pH 7.1 ± 0.2 at 25°C	

Precautions

1. For Laboratory Use.

User Quality Control**Identity Specifications**

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: Soluble in distilled or deionized water on boiling. (Add 1% glycerol after boiling). Light amber, slightly opalescent to opalescent, may have a precipitate.

Reaction of 6% Solution at 25°C : pH 7.1 ± 0.2 with 1% added glycerol and after autoclaving 5 minutes at $121\text{-}124^\circ\text{C}$.

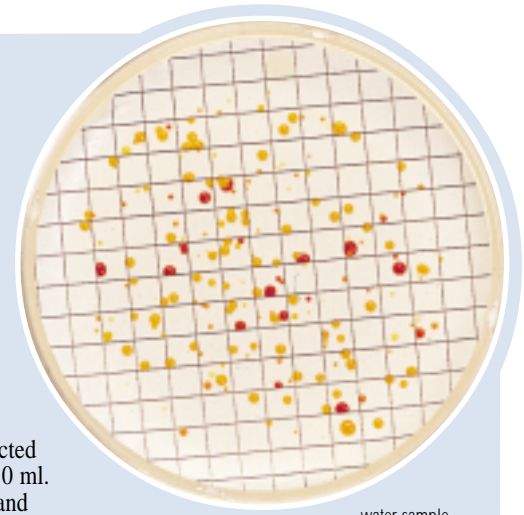
Prepared Plates: Light amber, opalescent, may have a precipitate.

Cultural Response

Prepare m HPC Agar per label instructions. Dilute ten chlorinated water samples collected as recommended by Standard Methods⁵ from different sources to yield 20-200 CFUs/10 ml. Filter the dilutions through a membrane filter. Place the filters on m-HPC Agar plates and incubate at $35 \pm 2^\circ\text{C}$ for 40-72 hours. Therecovery and morphology of bacteria on test medium should be comparable to that of a reference lot.

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.



water sample

- 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

m HPC Agar

Materials Required But Not Provided

Sterile Petri dishes, 50 x 9 mm

Membrane filter equipment

Dilution blanks

Pipettes or glass rods

Incubator (35°C)

Stereoscopic microscope

Sterile 47 mm, 0.45 µm, gridded membrane filters

Close fitting box or plastic bag containing moistened paper towels

Bacto Glycerol

Method of Preparation

- Suspend 6 grams in 100 ml distilled or deionized water.
- Heat to boiling to dissolve completely.
- Add 1 ml glycerol.
- Autoclave at 121-124°C for 5 minutes.
- Dispense 5 ml aliquots into Petri dishes.

Specimen Collection and Preparation

Water samples should be collected as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9060A.⁵

To minimize changes in bacterial population, water samples should be tested as soon as possible after collection. The recommended maximum elapsed time between collection and analysis of samples is 8 hours (maximum transit time of 6 hours, maximum processing time of 2 hours). When analysis cannot begin within 8 hours, maintain sample at a temperature below 4°C but do not freeze. Maximum elapsed time between collection and analysis must not exceed 24 hours.⁵

Test Procedure

- The volume to be filtered will vary with the sample. Select a maximum sample size to give 20 to 200 CFU per filter.
- Filter appropriate volume through a sterile 47 mm, 0.45 µm, gridded membrane filter, under partial vacuum. Rinse funnel with three 20 to 30 ml portions of sterile dilution water. Place filter on agar in Petri dish.
- Place dishes in close-fitting box or plastic bag containing moistened paper towels.
- Incubate at 35 ± 0.5°C for 48 hours. Duplicate plates may be incubated at other conditions as desired.

Results

Count all colonies on the membrane when there are 2 or less colonies per square. For 3 to 10 colonies per square, count 10 squares and obtain average count per square. For 10 to 20 colonies per square, count 5 squares and obtain average count per square. Multiply average count per square by 100 and divide by the sample volume to give colonies per milliliter. If there are more than 20 colonies per square, record count as > 2,000 divided by the sample volume. Report averaged counts as estimated colony-forming units. Make estimated counts only when there are discrete, separated colonies.⁵

Limitations of the Procedure

- m HPC Agar is intended for use only with the membrane filter method.
- m HPC Agar is recommended for testing treated water.
- Longer incubation times may be necessary to recover slow-growing bacteria.

References

- Taylor, R. H., and E. E. Geldreich.** 1979. A new membrane filter procedure for bacterial counts in potable water and swimming pool samples. *J. Amer. Water Works Assoc.* **71**:402-405.
- Means, E. G., L. Hanami, H. F. Ridgway, and B. H. Olson.** 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. *J. Amer. Water Works Assoc.* **73**:585-590.
- Nagy, L. A., and B. H. Olson.** 1982. The occurrence of filamentous fungi in drinking water distribution systems. *Can. J. Microbiol.* **28**:667-671.
- Haas, C. N., M. A. Meyer, and M. S. Paller.** 1982. Analytical note: evaluation of the m-SPC method as a substitute for the standard plate count in water microbiology. *J. Amer. Water Works Assoc.* **74**:322.
- 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.
- Lechevallier, M. W., R. J. Seidler, and T. M. Evans.** 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *App. And Environ. Microbiol.* **40**:922-930.
- Stapert, E. M., W. T. Sokolski, and J. I. Northam.** 1962. The factor of temperature in the better recovery of bacteria from water by filtration. *Can. Journal Microbiol.* **8**:809-810.
- Saleem, M., and R. L. Schlitzer.** 1983. Comparative recovery of bacteria from purified water by the membrane filter technique and the standard plate count methods, p. 281. *Abs. Ann. Meeting, ASM.*

Packaging

m HPC Agar	100 g	0752-15
	500 g	0752-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Heart Infusion Broth

Bacto Heart Infusion Agar

Intended Use

Bacto Heart Infusion Broth is used for cultivating fastidious microorganisms.

Bacto Heart Infusion Agar is an infusion medium used for cultivating a wide variety of fastidious microorganisms and as a base for preparing blood agar.

Also Known As

Heart Infusion Broth is abbreviated as HIB, Heart Infusion Agar as HIA.

Summary and Explanation

Heart Infusion Broth and Heart Infusion Agar are non-selective general purpose media used for the isolation of nutritionally fastidious microorganisms. One of the first media used for the cultivation of bacteria was a liquid medium containing an infusion of meat. Huntton¹ using fresh beef heart and Bacto Peptone, prepared a “hormone” broth to retain growth promoting substances. Highly pathogenic organisms, such as meningococci and pneumococci, could be grown on infusion medium without enrichments.¹ The formulas for HIA and HIB contain Tryptose, which is better suited to the nutritional requirements of pathogenic bacteria than Bacto Peptone.

Heart Infusion Agar can be used as a base for the preparation of blood agar in determining hemolytic reactions, and for mass cultivation of microorganisms in the preparation of vaccines. Heart Infusion Media are specified for the isolation of *Vibrio cholerae* and *Vibrio* species.^{2,3}

User Quality Control

Identity Specifications

Heart Infusion Broth

Dehydrated Appearance: Beige, homogeneous, free-flowing.

Solution: 2.5% solution, soluble in distilled or deionized water; light to medium amber in color, clear.

Prepared Medium: Light to medium amber, clear.

Reaction of 2.5% Solution at 25°C pH 7.4 ± 0.2

Heart Infusion Agar

Dehydrated Appearance: Beige, homogeneous, free-flowing.

Solution: 4% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent without significant precipitate.

Prepared Medium: Plain - Light to medium amber, slightly opalescent with no precipitate. With 5% sheep blood - cherry red, opaque.

Reaction of 4% Solution at 25°C pH 7.4 ± 0.2

continued on following page

Heart Infusion Broth may be used as the base in carbohydrate fermentation tests.⁴

Several modifications of Heart Infusion media have been described.⁵ The addition of carbohydrates, blood or other ingredients result in media used for a variety of purposes. The methodologies for the multiple applications using Heart Infusion Agar and Heart Infusion Broth are outlined in the references.

Principles of the Procedure

Infusion from Beef Heart and Tryptose supply the nutritional requirements for growth of microorganisms in Heart Infusion Media. Sodium chloride maintains the osmotic balance of the medium, and Bacto Agar is the solidifying agent. The addition of 5% sheep blood provides additional growth factors and is used to determine hemolytic reactions.

Formula

Heart Infusion Broth

Formula Per Liter	
Beef Heart, Infusion from	500 g
Bacto Tryptose	10 g
Sodium Chloride	5 g
Final pH 7.4 ± 0.2 at 25°C	

Heart Infusion Agar

Formula Per Liter	
Beef Heart, Infusion from	500 g
Bacto Tryptose	10 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.

Procedure

Materials Provided

Heart Infusion Broth
Heart Infusion Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile Petri dishes
Sterile tubes with closures

Method of Preparation**Heart Infusion Broth**

1. Dissolve 25 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Cool to room temperature.

Heart Infusion Agar

1. Suspend 40 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to Heart Infusion Agar at 45-50°C. Mix well.
4. Dispense into Petri dishes.

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.

Results

Refer to appropriate references and procedures for results.

References

1. **Huntoon, F. M.** 1918. "Hormone" Medium. A simple medium employable as a substitute for serum medium. *J. of Infect. Dis.* **23**:169-172.
2. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. p. 9.01-9.24. App. 3.24-3.25. *FDA Bacteriological Analytical Manual*, 8th ed. AOAC International, Arlington, VA.

*User Quality Control cont.***Cultural Response**

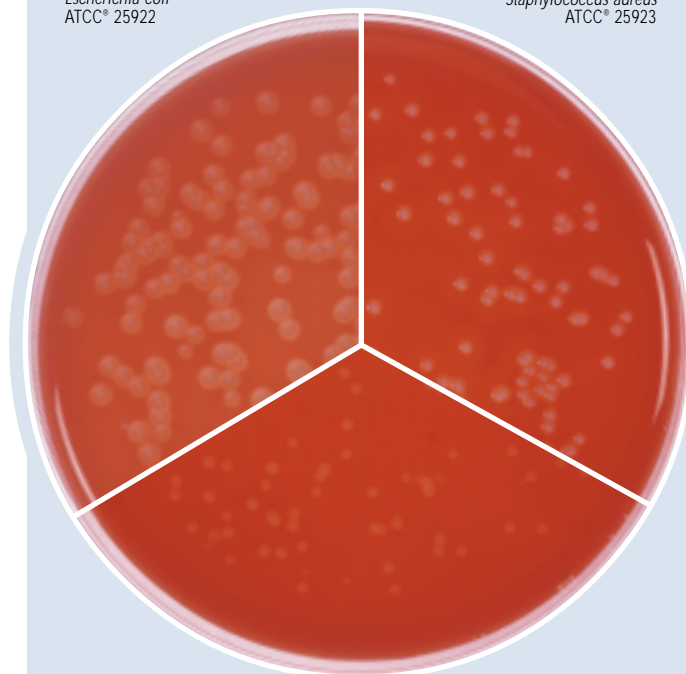
Prepare Heart Infusion Broth per label directions. Prepare Heart Infusion Agar with and without 5% sheep blood. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

Heart Infusion Broth

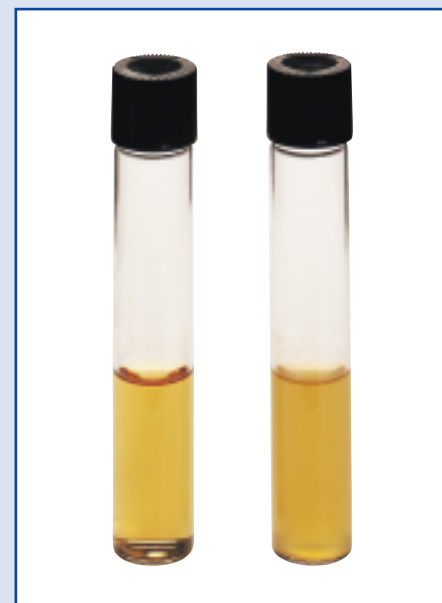
ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	25922*	100-1,000	good
<i>Staphylococcus aureus</i>	25923	100-1,000	good
<i>Streptococcus pneumoniae</i>	6305	100-1,000	good
<i>Streptococcus pyogenes</i>	19615*	100-1,000	good

Escherichia coli
ATCC® 25922

Staphylococcus aureus
ATCC® 25923



Streptococcus pyogenes ATCC® 19615
All with blood on Heart Infusion Agar



Uninoculated
tube

Escherichia coli
ATCC® 25922

Heart Infusion Agar

ORGANISM	ATCC*	INOCULUM CFU	GROWTH PLAIN	GROWTH w/5% SHEEP BLOOD	HEMOLYSIS w/5% SHEEP BLOOD
<i>Escherichia coli</i>	25922*	100-1,000	good	good	beta
<i>Staphylococcus aureus</i>	25923*	100-1,000	good	good	beta
<i>Streptococcus pneumoniae</i>	6305	100-1,000	fair	good	alpha
<i>Streptococcus pyogenes</i>	19615*	100-1,000	fair	good	beta

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.

- Vanderzant, C. and D. F. Splittstoesser (ed.). 1992. p. 451-469. 1132. Compendium of Methods for the Microbiological Examination of Food, 3rd ed. American Public Health Association, Washington, D.C.
- Ruoff, K. L. 1995. *Streptococcus*, p.305. In Murray, P.R., Baron E.J., Tenover, M.A., Tenover, F.C., and R.H. Tenover (ed.), Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
- Atlas, R. M. 1993. Handbook of Microbiological Media, p. 426-431, CRC Press, Boca Raton, FL.

Packaging

Heart Infusion Agar	100 g	0044-15
	500 g	0044-17
	2 kg	0044-07
	10 kg	0044-08
Heart Infusion Broth	100 g	0038-15
	500 g	0038-17
	2 kg	0038-07

Bacto® Hektoen Enteric Agar

Intended Use

Bacto Hektoen Enteric Agar is used for the isolating and differentiating gram-negative enteric bacilli.

Also Known As

Hektoen Enteric Agar is also known as HE Agar or HEA.

Summary and Explanation

Hektoen Enteric Agar was developed in 1967 by King and Metzger.^{1,2} Compared to other enteric differentiating media commonly used in clinical laboratories at that time, Hektoen Enteric Agar increased the frequency of isolation of *Salmonella* and *Shigella* organisms. This was accomplished by increasing the carbohydrate and peptone content of

the medium in order to counteract the inhibitory effects of the bile salts and indicators. King and Metzger formulated a medium that only slightly inhibited the growth of *Salmonella* and *Shigella* while at the same time ensuring the adequate inhibition of gram-positive microorganisms.

Hektoen Enteric Agar is used to isolate and differentiate *Salmonella* and *Shigella*, which cause a variety of serious human gastrointestinal illnesses.³ *Salmonella* is the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States.⁴ Foods containing poultry, eggs, or dairy products are the most frequent vehicles for foodborne salmonellosis. For food samples, a variety of procedures have been developed using Hektoen Enteric Agar as part of the multi-step procedure to isolate *Salmonella*.⁵⁻⁸

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light purplish beige, free-flowing, homogeneous.
Solution:	7.6% solution soluble in distilled or deionized water upon boiling.
Prepared Plates:	Green with yellowish cast, slightly opalescent.
Reaction of 7.6% Solution at 25°C:	pH 7.5 ± 0.2

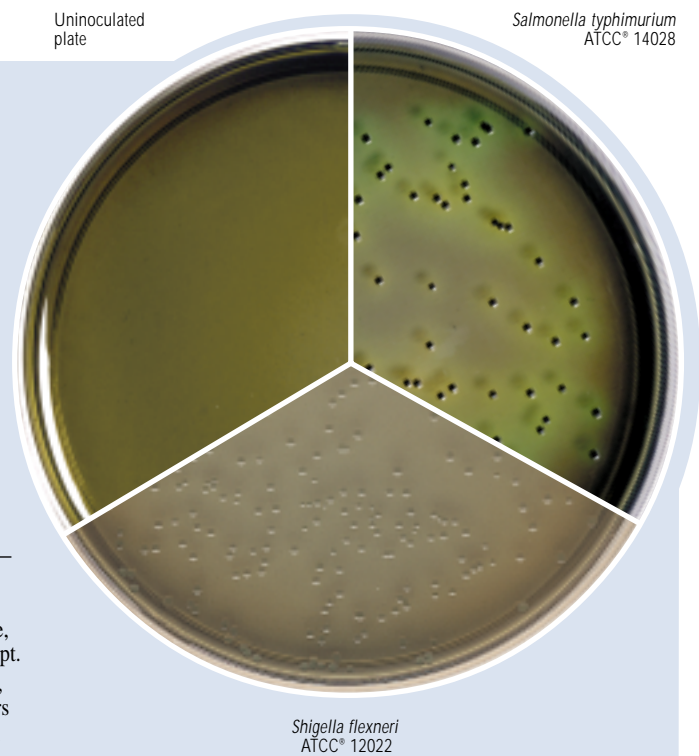
Cultural Response

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

ORGANISM	ATCC*	INOCULUM CFU	GROWTH	COLONY COLOR
<i>Enterococcus faecalis</i>	29212*	1,000-2,000	markedly inhibited	—
<i>Escherichia coli</i>	25922*	100-1,000	partial inhibition	salmon-orange, may have bile ppt.
<i>Salmonella typhimurium</i>	14028*	100-1,000	good	greenish blue, w/black centers
<i>Shigella flexneri</i>	12022*	100-1,000	good	greenish blue

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

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



Novobiocin (15 mg/liter) can be added to Hektoen Enteric Agar to inhibit growth of *Citrobacter* and *Proteus* colonies, which may resemble those of *Salmonella*.⁹

Principles of the Procedure

Proteose Peptone is a source of nitrogen and other nutrients in Hektoen Enteric Agar. Bile Salts and the dyes, brom thymol blue and acid fuchsin, inhibit gram-positive organisms. Lactose, saccharose and salicin are sources of fermentable carbohydrates. Ferric ammonium citrate, a source of iron, allows production of hydrogen sulfide (H₂S) from sodium thiosulfate. H₂S-positive colonies have black centers. Yeast Extract provides vitamins and cofactors required for growth and additional nitrogen and carbon. Bacto Agar is used as a solidifying agent.

Formula

Hektoen Enteric Agar

Formula Per Liter	
Bacto Proteose Peptone	12 g
Bacto Yeast Extract	3 g
Bacto Bile Salts No. 3	9 g
Bacto Lactose	12 g
Bacto Saccharose	12 g
Bacto Salicin	2 g
Sodium Chloride	5 g
Sodium Thiosulfate	5 g
Ferric Ammonium Citrate	1.5 g
Bacto Agar	14 g
Brom Thymol Blue	0.065 g
Acid Fuchsin	0.1 g
Final pH	7.5 ± 0.2 at 25°C

Precautions

- For Laboratory Use.
- 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.
- 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

Hektoen Enteric Agar

Materials Required but not Provided

Glassware
Autoclave
Incubator
Petri dishes

Method of Preparation

- Suspend 76 grams in 1 liter distilled or deionized water.
- Heat to boiling with frequent agitation to dissolve completely. Do not overheat. **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.

Limitations of the Procedure

- Do not autoclave this medium because excessive heat may alter the ingredients.
- Proteus* species may resemble salmonellae or shigellae. Further testing should be conducted to confirm the presumptive identification of organisms isolated on this medium.

References

- King, S., and W. I. Metzger.** 1968. A new plating medium for the isolation of enteric pathogens. *Appl. Microbiol.* **16**:577-578.
- King, S., and W. I. Metzger.** 1968. A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with SS and EMB Agar. *Appl. Microbiol.* **16**:579-581.
- Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella, and Yersinia*, p. 450-456. In Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
- Centers for Disease Control.** 1991. Summary of notifiable diseases. *Morbidity and Mortality Weekly Report*. **40** (53):3.
- Flowers, R. S., J-Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. In Vanderzant, C., and D. F. Splittstoesser (ed.), *Compendium of methods for the microbiological examination of foods*, 3rd ed. American Public Health Association, Washington, D.C.
- Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. In Marshall, R. T. (ed.), *Standard methods for the examination of dairy products*. 16th ed. American Public Health Association, Washington, D.C.
- Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. In *Bacteriological analytical manual*, 8th ed. AOAC International, Gaithersburg, MD.
- Association of Official Analytical Chemists.** 1996 official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.

9. **Hoben, D. A., D. H. Ashton, and A. C. Peterson.** 1973. Some observations on the incorporation of novobiocin into Hektoen Enteric Agar for improved *Salmonella* isolation. *Appl. Microbiol.* **26**:126-127.

Packaging

Hektoen Enteric Agar	100 g	0853-15
	500 g	0853-17
	2 kg	0853-07
	10 kg	0853-08

Bacto® Hemoglobin

Intended Use

Bacto Hemoglobin is used in preparing microbiological culture media.

Summary and Explanation

Hemoglobin, an autoclavable preparation of beef blood, is prepared according to the procedure described by Spray.¹

Hemoglobin is used with GC Medium Base is in the preparation of Chocolate Agar Enriched, Thayer-Martin Medium and Modified Thayer-Martin Medium. Supplemented with Hemoglobin and Supplement B or VX, the enriched media are used for the isolation and cultivation of fastidious microorganisms, especially *Neisseria* and *Haemophilus* species. With the exception of some laboratory-adapted strains of *Haemophilus aphrophilus*, *Haemophilus* species require either exogenous hemin (X factor), nicotinamide adenine dinucleotide (NAD) (V factor), or both.²

Principles of the Procedure

Hemoglobin provides the hemin (X factor) required for growth of *Haemophilus* and for enhanced growth of *Neisseria* species.

Formula

Hemoglobin is obtained from beef blood, desiccated.

Precautions

1. For Laboratory Use.

User Quality Control

Identity Specifications

Dehydrated Appearance: Dark brown, fine, free-flowing.
 Solution: 2% solution, insoluble in distilled or deionized water. Solution is chocolate brown, opaque with a dispersed precipitate.

Reaction of 2% Solution at 25°C: pH 8.2 ± 0.2

Cultural Response

Prepare GC Medium enriched with 2% Hemoglobin and Supplement B or VX per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO₂ for 18-48 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Haemophilus influenzae</i>	10211	100-1,000	good
<i>Neisseria gonorrhoeae</i>	43069	100-1,000	good

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

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

Storage

Store Hemoglobin below 30°C. The dehydrated ingredient 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

Hemoglobin

Materials Required But Not Provided

Glassware

Autoclave

GC Medium Base (for the cultivation of *Neisseria* and *Haemophilus* species)

Supplement B or VX, depending on the medium being prepared

Antimicrobial Vial CNV or CNVT, depending on the medium being prepared

Method of Preparation

1. Place 10 grams of Hemoglobin in a dry beaker.
2. Measure 500 ml distilled or deionized water.
3. Add the water in approximately 100 ml amounts, 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 the flask to reestablish complete solution, then add to an equal amount of double-strength sterile agar base cooled to 45-50°C.

Test Procedure

For a complete discussion on the isolation and identification of *Neisseria* and *Haemophilus* species, refer to procedures outlined in appropriate references.^{2,3,4}

Results

Refer to appropriate references and procedures for results.

References

1. **Spray.** 1930. *J. Lab Clin. Med.* **16**:166.
2. **Campos, J. M.** 1995. *Haemophilus*, p. 556-565. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.

3. **Isenberg, H. D. (ed.)**, 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. **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

Hemoglobin	100 g	0136-15
	500 g	0136-17
	2 kg	0136-07
	10 kg	0136-08

Bacto® Horse Serum, Desiccated

Intended Use

Bacto Horse Serum, Desiccated is used as an enrichment in bacteriological culture media.

Summary and Explanation

Horse Serum, Desiccated is an enrichment prepared from filter-sterilized, normal horse serum. When used in the preparation of Mycoplasma Supplement, Horse Serum supplies cholesterol, a growth stimulant for *Mycoplasma*.¹ Loeffler² used dextrose broth enriched with horse serum for cultivating *Corynebacterium diphtheriae*.

A medium supplemented with horse serum or lysed horse blood is usually sufficient to enhance the growth of fastidious anaerobes.³ Broth media supplemented with horse serum are used in the microdilution susceptibility testing of anaerobic bacteria.³

Principles of the Procedure

Horse Serum, Desiccated provides essential nutritional factors that stimulate organism growth.

Reagent

Horse Serum, Desiccated is sterile, lyophilized horse serum.

Precautions

1. For Laboratory Use.

User Quality Control

Identity Specifications

Lyophilized Appearance: Brown, lyophilized cake or powder.

Solution: Soluble in 10 ml distilled or deionized water.

Rehydrated Appearance: Light to medium amber, clear to slightly opalescent.

Cultural Response

Prepare Tryptose Blood Agar Base with 10% Horse Serum (rehydrated) per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Streptococcus mitis</i>	9895	100-1,000	good
<i>Streptococcus pneumoniae</i>	6303*	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.

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

Storage

Store Horse Serum, Desiccated and reconstituted Horse Serum 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

Horse Serum, Desiccated

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Horse Serum, Desiccated specified in the formula of the medium or enrichment being prepared. Add 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 using Horse Serum, Desiccated.¹⁻³

Results

Refer to appropriate references and procedures for results.

References

1. **Taylor-Robinson, D.** 1995. *Mycoplasma* and *Ureaplasma*, p. 652-661. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Loeffler, F.** 1887. Darauf theilte HeuLoeffler en einem Zweiten Vortrag die ergebnisse seiner weiteren untersuchungen uber die Diphtherie-Bacillen mit. Zentralb. Bacteriol. 2:105.
3. **Isenberg, H. D. (ed.)**, 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Horse Serum, Desiccated	12 x 10 ml	0261-61
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Bacto® ISP Medium 1 · Bacto ISP Medium 2

Bacto ISP Medium 4

Intended Use

Bacto ISP Medium 1, Bacto ISP Medium 2 and Bacto ISP Medium 4 are used for characterizing *Streptomyces* species according to the International Streptomyces Project (ISP).¹

User Quality Control

Identity Specifications

ISP Medium 1

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 0.8% solution, soluble in distilled or deionized water on boiling; light amber, clear to very slightly opalescent.

Prepared Medium: Light amber, clear to very slightly opalescent, w/o significant precipitation.

Reaction of 0.8% Solution at 25°C: pH 7.0 ± 0.2

ISP Medium 2

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 3.8% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.

Prepared Medium: Light to medium amber, slightly opalescent, without precipitate.

Reaction of 3.8% Solution at 25°C: pH 7.2 ± 0.2

ISP Medium 4

Dehydrated Appearance: White to light beige, free-flowing, homogeneous.

Solution: 3.7% solution, soluble in distilled or deionized water on boiling; white to off-white, opaque with precipitate.

Prepared Medium: White to off-white, opaque, may have a precipitate.

Reaction of 3.7% Solution at 25°C: pH 7.2 ± 0.2

Cultural Response

Prepare ISP Medium 1, ISP Medium 2 and ISP Medium 4 per label directions. Inoculate tubes of prepared ISP Medium 1, and incubate at 30 ± 2°C for 48-96 hours.

Inoculate prepared ISP Medium 2 and ISP Medium 4 with the test organisms by placing a drop of inoculum near the edge of the plate. Five parallel streaks across the plate are made from this drop, followed by four perpendicular streaks. Incubate inoculated plates at 30 ± 2°C for 48-96 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Streptomyces albus</i>	3004	100-1,000	good
<i>Streptomyces lavendulae</i>	8664	100-1,000	good

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

Also Known As

ISP Medium 1 is also referred to as Tryptone Yeast Extract Broth.

ISP Medium 2 is also referred to as Yeast Malt Extract Agar.

ISP Medium 4 is also referred to as Inorganic Salts Starch Agar.

Summary and Explanation

ISP media were developed by Difco Laboratories for the International Streptomyces Project (ISP) in order to select stable properties and reproducible procedures for characterization of *Streptomyces* species.¹

Principles of the Procedure

Tryptone and Yeast Extract are the nitrogen, vitamin, carbon and amino acid source in ISP Medium 1.

Yeast Extract and Malt Extract provide nitrogen, amino acids and vitamins in ISP Medium 2. Dextrose is the carbon source, and Bacto Agar is the solidifying agent.

ISP Medium 4 is composed of many inorganic salts and Soluble Starch to provide essential nutrients for organism growth. Bacto Agar is the solidifying agent.

Formula

ISP Medium 1

Formula Per Liter	
Bacto Tryptone	5 g
Bacto Yeast Extract	3 g
Final pH 7.0 ± 0.2 at 25°C	

ISP Medium 2

Formula Per Liter	
Bacto Yeast Extract	4 g
Bacto Malt Extract	10 g
Bacto Dextrose	4 g
Bacto Agar	20 g
Final pH 7.2 ± 0.2 at 25°C	

ISP Medium 4

Formula Per Liter	
Bacto Soluble Starch	10 g
Potassium Phosphate, Dibasic	1 g
Magnesium Sulfate USP	1 g
Sodium Chloride	1 g
Ammonium Sulfate	2 g
Calcium Carbonate	2 g
Ferrous Sulfate (FeSO ₄ ·7H ₂ O)	0.001 g
Manganous Chloride (MnCl ₂ ·7H ₂ O)	0.001 g
Zinc Sulfate (ZnSO ₄ ·7H ₂ O)	0.001 g
Bacto Agar	20 g
Final pH 7.2 ± 0.2 at 25°C	

Precautions

- For Laboratory Use.

2. ISP Medium 4

IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breath dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Intestines.

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.

- 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**

ISP Medium 1
ISP Medium 2
ISP Medium 4

Materials Required But Not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (30°C)
Sterile tubes
Sterile Petri dishes

Method of Preparation

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

ISP Medium 1	8 g/l
ISP Medium 2	38 g/l
ISP Medium 4	37 g/l
- Heat to boiling to dissolve completely.
- Autoclave at 121°C for 15 minutes.
- Mix thoroughly while dispensing.

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 maintenance of *Streptomyces* species refer to appropriate references.^{2,3}

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

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

References

- Shirling, E. B., and D. Gottlieb.** 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16**:313-340.
- Isenberg, H. D. (ed.).** 1992. *Clinical microbiology procedures handbook.* American Society for Microbiology, Washington, D.C.
- Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.).** 1995. *Manual of clinical microbiology, 6th ed.* American Society for Microbiology, Washington, D.C.

Packaging

ISP Medium 1	500 g	0769-17
ISP Medium 2	500 g	0770-17
ISP Medium 4	500 g	0772-17

Bacto® Inositol Assay Medium**Intended Use**

Bacto Inositol Assay Medium is used for determining inositol concentration by the microbiological assay technique.

Summary and Explanation

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

- Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
- Inoculum Media: To condition the test culture for immediate use;
- Assay Media: To permit quantitation of the vitamin under test.

Inositol Assay Medium, a modification of the formula described by Atkin et al.,¹ is used in the microbiological assay of inositol using

Saccharomyces cerevisiae ATCC® 9080 (*Saccharomyces uvarum*) as the test organism.

Principles of the Procedure

Inositol Assay Medium is an inositol-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *S. cerevisiae* ATCC® 9080. The addition of inositol in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula**Inositol Assay Medium**

Formula Per Liter	
Bacto Dextrose	100 g
Potassium Citrate	10 g
Citric Acid	2 g

Monopotassium Phosphate	1.1 g
Potassium Chloride	0.85 g
Magnesium Sulfate	0.25 g
Calcium Chloride	0.25 g
Manganese Sulfate	50 mg
Ferric Chloride	50 mg
DL-Tryptophane	80 mg
L-Cystine	0.1 g
L-Isoleucine	0.5 g
L-Leucine	0.5 g
L-Lysine	0.5 g
L-Methionine	0.2 g
DL-Phenylalanine	0.2 g
L-Tyrosine	0.2 g
L-Asparagine	0.8 g
DL-Aspartic Acid	0.2 g
DL-Serine	0.1 g
Glycine	0.2 g
DL-Threonine	0.4 g
L-Valine	0.5 g
L-Histidine	0.124 g
L-Proline	0.2 g
DL-Alanine	0.4 g
L-Glutamic Acid	0.6 g
L-Arginine	0.48 g
Thiamine Hydrochloride	500 µg
Biotin	16 µg
Calcium Pantothenate	5 mg
Pyridoxine Hydrochloride	1 mg
Final pH 5.2 ± 0.2 at 25°C	

Precautions

1. For Laboratory Use.
2. Great care must be taken to avoid contamination of media or glass-

User Quality Control

Identity Specifications

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	6.1% (single strength) or 12.2% (double strength) solution, soluble in distilled or deionized water on boiling. Light amber, clear, may have a slight precipitate.
Prepared Medium:	Light amber, clear, may have a slight precipitate.
Reaction of 6.1% Solution at 25°C:	pH 5.2 ± 0.2

Cultural Response

Prepare Inositol Assay Medium per label directions. Dispense medium into 50 ml flasks with a titration from 0 to 10 µg of Inositol. Inoculate flasks with one drop of *S. cerevisiae* ATCC® 9080 inoculum suspension (washed three times and diluted 1:1000). Incubate flasks at 25-30°C for 20-24 hours. The curve obtained from turbidimetric readings should be typical.

ware 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 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

Inositol Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Saccharomyces cerevisiae* ATCC® 9080
Inositol
Sterile tubes
Sterile 0.85% saline
Distilled or deionized water
Lactobacilli Agar AOAC
Centrifuge
Spectrophotometer

Method of Preparation

1. Suspend 12.2 grams in 100 ml distilled or deionized water.
2. Boil to dissolve.
3. Dispense 5 ml amounts into flasks.
4. Add standard or test samples.
5. Adjust flask 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. The samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Remove a loopful of culture from a stock culture slant of *S. cerevisiae* ATCC® 9080 and suspend it in 10 ml sterile 0.85% saline. Centrifuge cells at moderate speed for 10 minutes. Decant the supernatant and resuspend cells in 10 ml 0.85% sterile saline. Wash the cells three times with 10 ml sterile 0.85% saline. After the third wash, resuspend the

Intended Use

Bacto KF Streptococcus Agar is used with Bacto TTC Solution 1% in isolating and enumerating fecal streptococci according to APHA.

Also Known As

Kenner Fecal Streptococcus Agar

Summary and Explanation

Kenner et al. developed KF Streptococcal Agar for use in detecting streptococci in surface waters by direct plating or by the membrane filtration method.¹ These investigators compared the performance of their formulation to other media used for enumerating fecal streptococci and achieved greater recoveries with KF Streptococcal Agar.

This medium is currently recommended for use in determining counts of fecal streptococci in foods and water.^{2,3}

Principles of the Procedure

Peptone provides a source of nitrogen, amino acids and carbon. Yeast Extract is a source of trace elements, vitamins and amino acids. Maltose and Lactose are fermentable carbohydrates and carbon sources. Sodium Azide is a selective agent. Brom Cresol Purple is an indicator dye.

The addition of 1% triphenyltetrazolium chloride (TTC) causes enterococci to develop a deep red color following reduction of tetrazolium to an acid azo dye.

Formula

KF Streptococcus Agar

Formula Per Liter	
Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	10 g
Sodium Chloride	5 g
Sodium Glycerophosphate	10 g
Bacto Maltose	20 g
Bacto Lactose	1 g
Sodium Azide	0.4 g
Bacto Brom Cresol Purple	0.015 g
Bacto Agar	20 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 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

KF Streptococcus Agar
TTC Solution 1%

Materials Required But Not Provided

Glassware
Incubator (35°C)
Pipettes
Sterile Petri dishes, 50 x 9 mm
Membrane filter equipment
Sterile 47 mm, 0.45 µm, gridded membrane filters
Dilution blanks
Stereoscopic microscope

Method of Preparation

1. Suspend 76.4 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
2. Heat an additional 5 minutes. Avoid overheating which could decrease the productivity of the medium. DO NOT AUTOCLAVE.
3. Add 10 ml TTC Solution 1% to the medium at 50°C and mix well.
4. Pour medium into sterile Petri dishes if using the Membrane Filter procedure. If using the Pour Plate technique, hold the liquid medium at 45°C.

Specimen Collection and Preparation

Consult appropriate references for specific procedures using KF Streptococcus Agar in the examination of waters and food.^{2,3} The pour plate technique or the membrane filter procedure can be used for detection and enumeration of enterococci.

Test Procedure

Pour Plate Technique

1. Prepare appropriate dilutions of the test material.
2. Place the selected volume of sample in a Petri dish.
3. Pour 15 ml of the prepared medium at 45°C into each plate.
4. Thoroughly mix the medium and sample to uniformly disperse the organisms.
5. Allow the agar to solidify.
6. Incubate plates in the inverted position at 35 ± 2°C for 46-48 hours.

Membrane Filter Procedure

1. Filter a suitable volume of sample through a sterile membrane, as directed.
2. Place the inoculated membrane filter on the solidified agar in the Petri dish, inoculum side up.
3. Incubate the plates, inverted, at 35 ± 2°C for 46-48 hours.

Formula

KF Streptococcus Broth

Formula Per Liter	
Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	10 g
Sodium Chloride	5 g
Sodium Glycerophosphate	10 g
Bacto Maltose	20 g
Bacto Lactose	1 g
Sodium Azide	0.4 g
Bacto Brom Cresol Purple	0.015 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

KF Streptococcus Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Pipettes
Culture tubes
Membrane filter equipment
TTC Solution 1%
Incubator (35°C), saturated with water vapor
Sterile Petri dishes, 50 x 9 mm
Sterile 47 mm, 0.45 µm, gridded membrane filters
Sterile absorbent pads
Stereoscopic microscope

Method of Preparation

MPN Procedure

1. For an inoculum of 1 ml or less, suspend 56.4 g in 1 liter distilled or deionized water.
For an inoculum of 10 ml, suspend 84.6 g in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. For an inoculum of 1 ml or less, dispense 10 ml amounts into culture tubes.
For an inoculum of 10 ml, dispense 20 ml amounts into culture tubes.
4. Autoclave at 121°C for 10 minutes.

Membrane Filter Procedure

1. Suspend 56.4 g in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 100 ml amounts into flasks and autoclave at 121°C for 10 minutes.
4. Cool to 60°C.
5. Add 1 ml TTC Solution 1% per 100 ml of medium.

Specimen Collection and Preparation

Water or food samples should be collected and prepared according to appropriate references.

Test Procedure

MPN Procedure

1. Inoculate tubes of the KF Streptococcus Broth with the appropriate amount of inoculum.
2. Incubate tubes at 35 ± 1°C, with loosened caps, for 46-48 hours.

Membrane Filter Procedure

1. Place a sterile absorbent pad in each sterile Petri dish.
2. Saturate the pads with the sterile medium containing TTC.
3. Place an inoculated membrane filter, inoculated side up, on the saturated pad.
4. Incubate at 35 ± 1°C in an atmosphere saturated with water vapor for 46-48 hours.

Results

MPN Procedure

MPN tubes positive for enterococci are turbid with growth that appears yellow in color and does not produce foaming. When foaming occurs, confirmation for enterococci should be made by Gram staining.

Membrane Filter Procedure

All red or pink colonies visible with 15x magnification are counted as enterococci colonies.

Limitations of the Procedure

1. Many strains of *S. bovis* and *S. equinus* are inhibited by azide.
2. The pH of KF Streptococcus Broth should be between 7.2 and 7.3. If below 7.0, it should not be used.
3. Overheating may lower the pH, resulting in a decrease in productivity of the medium.

References

1. **Kenner, B. A., H. F. Clark, and P. W. Kabler.** 1960. Fecal streptococci. II. Quantification of streptococci in feces. *Am. J. Public Health* **50**:1553.
2. **Kenner, B. A., H. F. Clark, and P. W. Kabler.** 1961. Fecal streptococci. I. Cultivation and enumeration of streptococci in surface waters. *Appl. Microbiol.* **9**:15.
3. **MacFadden, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkens, Baltimore, MD.
4. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. *Appl. Microbiol.* **20**:245-250.
5. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

KF Streptococcus Broth 500 g 0997-17

KL Virulence Agar

Bacto® KL Virulence Agar · KL Virulence Enrichment KL Antitoxin Strips

Intended Use

Bacto KL Virulence Agar is used with Bacto KL Virulence Enrichment, Bacto Chapman Tellurite Solution 1% and Bacto KL Antitoxin Strips in differentiating virulent (toxigenic) from nonvirulent strains of *Corynebacterium diphtheriae*.

Also Known As

KL Virulence Agar conforms with Klebs-Loeffler Virulence Agar.

Summary and Explanation

Elek² was the first to describe the agar plate diffusion technique for demonstrating the in vitro toxigenicity (virulence) of *Corynebacterium diphtheriae*. King, Frobisher, and Parsons³ expanded on Elek's technique and, by using a carefully standardized medium, obtained results in agreement with animal inoculation tests. These authors demonstrated that Difco Proteose Peptone possessed properties essential for toxin production. Incorporating Difco Proteose Peptone into the test medium assured consistent results. The authors used rabbit, sheep and horse serum as enrichments, finding human serum to be unsatisfactory. To overcome irregularities encountered in previous formulations, Hermann, Moore, and Parsons¹ refined the medium used for the in vitro KL Virulence Test, simplifying the basal medium and developing a nonserous enrichment. The medium and enrichment described by these authors have been standardized for use in the KL Virulence Test.

KL Virulence Agar and KL Virulence Enrichment are prepared according to the formulation of Hermann, Moore and Parsons.¹

Principles of the Procedure

Proteose Peptone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms and for toxin production. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is incorporated as a solidifying agent. KL Virulence Enrichment, composed of Casamino Acids, Glycerol and Tween® 80, provides a source of nonserous enrichment. Casamino

Acids is derived from acid-hydrolyzed casein that has low sodium chloride and iron concentrations. The low iron concentration is beneficial because iron is known to prevent the production of diphtheria toxin when present in more than minute amounts. Glycerol (glycerine) contains no heavy metals and is used by bacteria as a source of carbon. Tween® 80 improves growth of certain strains of *Corynebacterium diphtheriae*. Toxin produced by bacteria and diffused into the medium is detected by precipitation with the antitoxin present on the KL Antitoxin Strip. Chapman Tellurite Solution 1% (1% potassium tellurite solution) inhibits gram-negative and most gram-positive bacteria except *Corynebacterium* spp., *Streptococcus mitis*, *S. salivarius*, enterococci, and possibly *Staphylococcus epidermidis*. This permits direct testing of mixed primary cultures.

Formula

KL Virulence Agar

Formula Per Liter
 Bacto Proteose Peptone 20 g
 Sodium Chloride 2.5 g
 Bacto Agar 15 g
 Final pH 7.8 ± 0.2 at 25°C

KL Virulence Enrichment

Formula Per 100 ml
 Bacto Casamino Acids 1 g
 Glycerol 1 ml
 Tween® 80 1 ml

KL Antitoxin Strips

KL Antitoxin Strips are 1 x 7 cm filter paper strips containing antitoxin to diphtheria toxin.

Precautions

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

Storage

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

Store KL Virulence Enrichment and KL Antitoxin Strips at 2-8°C.

Store prepared plates 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

KL Virulence Agar
KL Virulence Enrichment
KL Antitoxin Strips

Materials Required But Not Provided

Glassware
Autoclave
Water bath (55-60°C)
Incubator (35°C)
Chapman Tellurite Solution 1%

Method of Preparation

1. Suspend 37.5 grams of KL Virulence Agar in 1 liter distilled or deionized water and heat to boiling to dissolve completely.
2. Autoclave at 121°C for 15 minutes.
3. Cool in a water bath to 55-60°C.
4. Aseptically dispense 10 ml of KL Virulence Agar into a Petri dish containing 2 ml KL Virulence Enrichment and 0.5 ml Chapman Tellurite Solution 1%; mix thoroughly.
5. Using aseptic technique, submerge a KL Antitoxin Strip or equivalent beneath the agar prior to solidification.

Specimen Collection and Preparation

For cases of suspected diphtheria, material for culture is obtained on a swab from the inflamed membranes of the throat and nasopharynx, or from wounds.⁴ Care must be taken not to contaminate the swab with normal skin flora. The specimen should be immediately transported to a laboratory and inoculated onto the proper media. If the specimen is to be shipped to a laboratory, it should be placed in a sterile tube or a special packet containing a desiccant such as silica gel.⁵

User Quality Control

Identity Specifications

KL Virulence Agar

Dehydrated Appearance: Light beige with some small dark specks, free-flowing, homogeneous.

Solution: 3.75% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent, with a slight precipitate.

Reaction of 3.75% Solution at 25°C: pH 7.8 ± 0.2

Prepared Medium: Light medium amber, slightly opalescent, may have a slight precipitate.

KL Virulence Enrichment

Appearance: Colorless to very light amber, clear liquid.

KL Antitoxin Strips

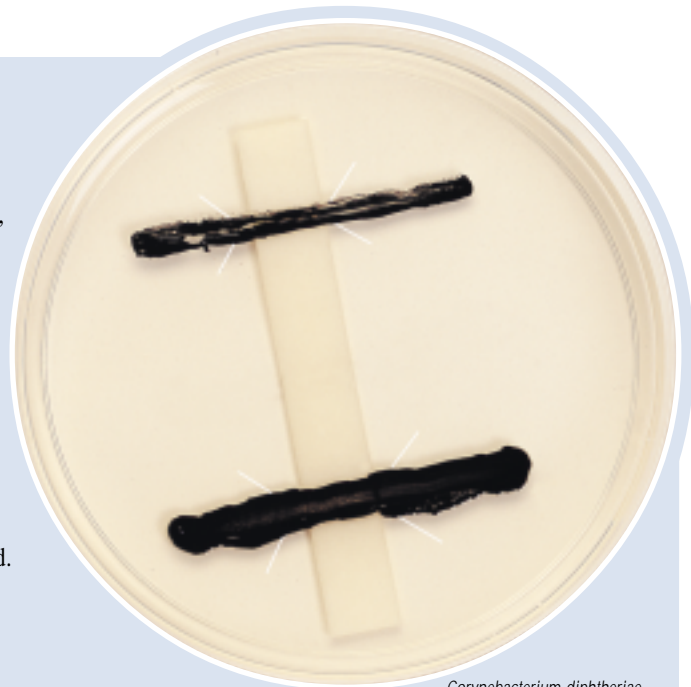
Appearance: White, filter paper strips, 1 x 7 cm.

Cultural Response

Prepare KL Virulence Agar per label directions, including KL Virulence Enrichment, Chapman Tellurite Solution 1% and one KL Antitoxin Strip per plate. Inoculate and incubate at 35 ± 2°C under CO₂ for up to 72 hours.

ORGANISM	ATCC*	GROWTH
<i>Corynebacterium diphtheriae</i> Type <i>gravis</i>	8028	+
<i>Corynebacterium diphtheriae</i> Type <i>intermedius</i>	8032	+
<i>Staphylococcus aureus</i>	25923*	-

+ = positive, line of precipitation at 45° angle to the strip
- = negative, no line of precipitation



Corynebacterium diphtheriae
ATCC® 8028

Precipitate lines are graphically enhanced for demonstration purposes (see Results).

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

*This organism is available as a Bactrol™ culture and should be used as directed.

Test Procedure

Inoculate the medium by streaking a loopful of a 24-hour culture in a single line across the plate perpendicular to (right angle to) the antitoxin strip. (Do not touch the actual strip itself). As many as eight cultures may be tested on a single plate.⁶ Place test isolates about 1 cm apart. Also inoculate a toxigenic (positive control) and a nontoxigenic (negative control) *C. diphtheriae* strain approximately 1 cm on either side of the test isolates.⁶ Incubate the inverted plates at 37°C for 72 hours. Examine at 24-, 48- and 72-hour intervals.

Results

Toxigenic (virulent) cultures of *C. diphtheriae* will show fine lines of precipitation at approximately 45° angles from the culture streak. This line forms where toxin (from the bacteria) combines with antitoxin from the strip. Primary precipitin lines form an arc of identity with the precipitin line produced by an adjacent positive control strain.⁷ Nontoxigenic strains of *C. diphtheriae* will show no lines of precipitation.

Limitations of the Procedure

1. Each test should include positive and negative controls.⁵
2. False-positive reactions may be seen after 24 hours as weak bands near the antitoxin strip. These can be recognized when compared with the positive control.⁸
3. *Corynebacterium ulcerans* and *C. pseudotuberculosis* may also produce lines of toxin-antitoxin.⁹

References

1. **Hermann, G. J., M. S. Moore, and E. I. Parsons.** 1958. A substitute for serum in the diphtheria in vitro test. *Am. J. Clin. Pathol.* **29**:181-183.
2. **Elek, S. D.** 1948. The recognition of toxicogenic bacterial strains in vitro. *Brit. Med. J.* **1**:493.

3. **King, E. O., M. Frobisher, Jr., and E. I. Parsons.** 1949. The in vitro test for virulence of *Corynebacterium diphtheriae*. *Am. J. Public Health* **39**:1314.
4. **Clarridge, J. E., and C. A. Spiegel.** 1995. *Corynebacterium* and miscellaneous irregular gram-positive rods, *Erysipelothrix* and *Gardnerella*, p. 357-378. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Krech, T., and D. G. Hollis.** 1991. *Corynebacterium* and related organisms, p. 277-286. In A. Ballows, W. J. Hausler, Jr., K. Herrmann, H. D. Isenberg, and H. J. Shadomy (eds.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 410-414. Williams & Wilkins, Baltimore, MD.
7. **Washington, J. A., Jr.** 1981. *Laboratory procedures in clinical microbiology*. Springer-Verlag, New York, NY.
8. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and J. P. Truant (eds.)**. 1980. *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
9. **Branson, D.** 1972. *Methods in clinical bacteriology*. Charles C. Thomas, Springfield, IL.

Packaging

KL Virulence Agar	500 g	0985-17
KL Virulence Enrichment	12 x 20 ml	0986-64
KL Antitoxin Strips	12 strips	3101-30
Chapman Tellurite Solution 1%	6 x 1 ml	0299-51
	6 x 25 ml	0299-66

Bacto® Kligler Iron Agar

Intended Use

Bacto Kligler Iron Agar is used for differentiating pure cultures of gram-negative bacilli based on the fermentation of dextrose and lactose and production of hydrogen sulfide.

Also Known As

Kligler Iron Agar is also known as KIA.

Summary and Explanation

Kligler Iron Agar is a modification of Kligler's¹ original formula. It is recommended to identify pure cultures of colonies picked from primary plating media, such as MacConkey Agar. Kligler's¹ original medium was a soft nutrient agar containing dextrose, Andrade indicator and lead acetate. Russell² devised a medium containing glucose, lactose, and an indicator for the differentiation of lactose-fermenting and nonlactose-fermenting gram negative bacilli. Kligler³ found that lead acetate for the detection of hydrogen sulfide

could be successfully combined with Russell double sugar medium for the differentiation of the typhoid, paratyphoid and dysentery groups. Bailey and Lacy⁴ simplified the formula by using phenol red as the pH indicator instead of Andrade indicator. A similar medium containing saccharose, Tryptone, ferrous sulfate and thiosulfate was developed by Sulkin and Willett.⁵

Kligler Iron Agar is recommended for differentiation of enteric gram-negative bacilli from clinical specimens⁶⁻⁸ and food samples.^{9,10}

Principles of the Procedure

Kligler Iron Agar combines the principles of Russell double sugar agar and lead acetate agar into one medium. This combination permits the differentiation of the gram-negative bacilli both by their ability to ferment dextrose or lactose and to produce hydrogen sulfide. Beef Extract, Yeast Extract, Bacto Peptone, and Proteose Peptone provide nitrogen, vitamins and minerals. Ferrous sulfate and sodium thiosulfate are the indicators of hydrogen sulfide production. Phenol red is the pH indicator. Sodium chloride maintains the osmotic balance of the medium. Bacto® Agar is the solidifying agent.

Formula

Kligler Iron Agar

Formula Per Liter	
Bacto Beef Extract	3 g
Bacto Yeast Extract	3 g
Bacto Peptone	15 g
Bacto Proteose Peptone	5 g
Bacto Lactose	10 g
Bacto Dextrose	1 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Bacto Agar	12 g
Bacto Phenol Red	0.024 g
Final pH 7.4 ± 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 tubes at 2-8°C.

Expiration Date

The expiration date applies to the medium 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

Kligler Iron Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Tubes with closures
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 55 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes with closures.
4. Autoclave at 121°C for 15 minutes. Cool in slanted position with deep butts.

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.⁶⁻¹⁰
2. Process each specimen, using procedures appropriate for that specimen or sample.⁶⁻¹⁰

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish beige, free flowing, homogeneous.
Solution:	5.5% solution; soluble in distilled or deionized water on boiling. Orange-red, slightly opalescent with precipitate.
Prepared Tubes:	Slightly orange-red, slightly opalescent, slight precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

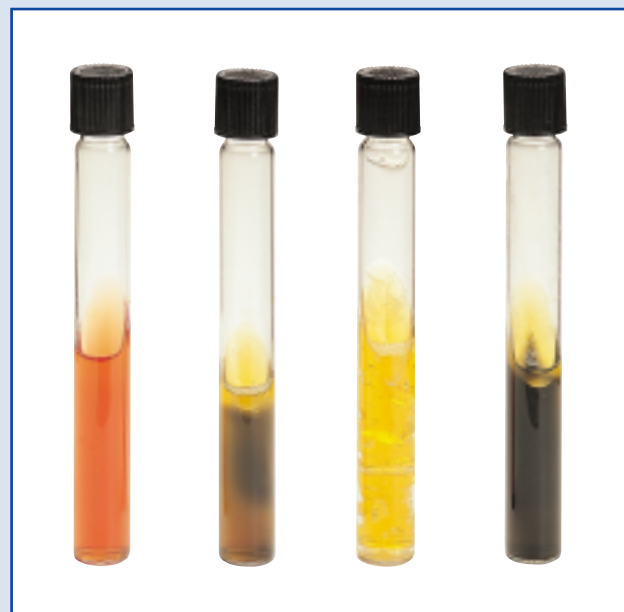
Prepare Kligler Iron Agar per label directions. Inoculate and incubate tubes at 35°C for 18-48 hours.

ORGANISM	ATCC*	CFU	GROWTH	SLANT/ BUTT	GAS	H ₂ S
<i>Citrobacter freundii</i>	8090*	undiluted	good	A/A	+	+
<i>Escherichia coli</i>	25922*	undiluted	good	A/A	+	-
<i>Proteus vulgaris</i>	6380	undiluted	good	K/A	-	+

A = acid reaction (yellow) K = alkaline reaction (no color change)
+gas = cracks, splits or bubbles in medium -gas = no cracks, splits, or bubbles in medium
+H₂S = black precipitate in butt -H₂S = no black precipitate in butt

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 *Citrobacter freundii* ATCC® 8090 *Escherichia coli* ATCC® 25922 *Proteus vulgaris* ATCC® 6380

Bacto® LB Agar, Lennox

Intended Use

Bacto LB Agar, Lennox is used for maintaining and cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

LB Agar, Lennox, a nutritionally rich medium, was developed by Lennox for the growth and maintenance of pure cultures of recombinant strains of *E. coli*.¹ These strains are generally derived from *E. coli* K12, which are deficient in B vitamin production. This strain of *E. coli* has been further modified through specific mutation to create an auxotrophic strain that is not capable of growth on nutritionally deficient media. LB Agar, Lennox provides all the nutritional requirements of these organisms. LB Agar, Lennox contains half the sodium chloride level of the Miller formulation of LB Agar.² This allows the researcher to select the optimal salt concentration for a specific strain.

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride. Bacto Agar is the solidifying agent.

Formula

LB Agar, Lennox

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is light beige, slightly opalescent.
Prepared Plates:	Medium amber, very slightly to slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

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

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	23724	100-300	Good
<i>Escherichia coli</i>	33694	100-300	Good
<i>Escherichia coli</i>	33849	100-300	Good
<i>Escherichia coli</i>	39403	100-300	Good
<i>Escherichia coli</i>	47014	100-300	Good
<i>Escherichia coli</i>	53868	100-300	Good

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

Sodium Chloride	5 g
Bacto Agar	15 g
Final pH	7.0 ± 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 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

LB Agar, Lennox

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

Specimen Collection and Preparation

Not applicable.

Test Procedure

Consult appropriate references for recommended test procedures.²

Results

After sufficient incubation, the medium should show growth as evidenced by formation of colonies and/or a confluent lawn of growth.

References

1. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**:190.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1994. Current protocols in molecular biology, vol. 1. Current Protocols, New York, N.Y.

Packaging

LB Agar, Lennox 500 g 0401-17

Bacto® LB Agar, Miller

Intended Use

Bacto LB Agar, Miller is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures.

Summary and Explanation

LB Agar, Miller is based on LB Medium as described by Miller for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures.¹⁻³ LB Agar, Miller is a nutritionally rich medium designed for growth of pure cultures of recombinant strains. *E. coli* grows more rapidly on this rich medium because it provides the cells with amino acids, nucleotide precursors, vitamins and other metabolites that the microorganism would otherwise have to synthesize.⁴

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by sodium chloride. Agar is added to the medium as a gelling agent.

Formula

LB Agar, Miller

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	10 g
Bacto Agar	15 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.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light to light tan, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in distilled or deionized water on boiling. Solution is very light amber, slightly opalescent.
Prepared Plates:	Very light amber, slightly opalescent.
Reaction of 4.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Bacto LB Agar, Miller per label directions. Inoculate plates and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	33526	100-1000	Good

The culture listed is the minimum that should be used for performance testing.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium 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

LB Agar, Miller

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 40 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Not applicable.

Test Procedure

Consult appropriate references for recommended test procedures.³⁻⁶

Results

Growth should be evident by the appearance of colonies and/or a confluent lawn on the surface of the medium.

References

1. **Luria, S. E., and J. W. Burrous.** 1955. Hybridization between *Escherichia coli* and *Shigella*. *J. Bacteriol.* **74**:461-476.
2. **Luria, S. E., J. N. Adams, and R. C. Ting.** 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology* **12**:348-390.
3. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1994. Current protocols in molecular biology. Greene Publishing Associates, Inc., Brooklyn, NY.
5. **Sambrook, J., E. F. Fitch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd. ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
6. **Lennox, E. S.** 1955. Transduction of linked genetic character of the host by bacteriophage P1. *Virology* **1**:190-206.

Packaging

LB Agar, Miller	500 g	0445-17
	2 kg	0445-07

Bacto® LB Broth, Lennox

Intended Use

Bacto LB Broth, Lennox is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures.

Summary and Explanation

LB Broth, Lennox is a nutritionally rich medium designed for growth of pure cultures of recombinant strains. The formula is based on L Broth described by Lennox for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures.¹ *E. coli* is grown to late log phase in LB Broth. Some plasmid vectors may replicate to high copy numbers without selective amplification. Some vectors may require selective amplification to reach high copy numbers. Chloramphenicol can be added to inhibit host synthesis and, as a result, prevent replication of the bacterial chromosome.²

LB Broth, Lennox contains ten times the sodium chloride level of Luria Broth Base, Miller and one half of that found in LB Broth, Miller.³ This allows the researcher to select the optimal salt concentration for a specific strain. If desired, the medium may be aseptically supplemented with glucose to prepare the complete medium described by Lennox.

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride.

Formula

LB Broth, Lennox

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g
Final pH 7.0 ± 0.2 at 25°C	

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in distilled or deionized water. Solution is very light amber, clear to very slightly opalescent.
Prepared Medium:	Very light amber, clear to very slightly opalescent.
Reaction of 2.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare LB Broth, Lennox per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	53868 (DH5)	100-300	Good
<i>Escherichia coli</i>	JM103	100-300	Good
<i>Escherichia coli</i>	33694 (HB101)	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 the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

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

LB Broth, Lennox

Materials Required But Not Provided

Flasks with closures
Tubes with closures
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 20 grams in 1 liter of distilled or deionized water.
2. Dispense into tubes with closures.
3. Autoclave at 121°C for 15 minutes.
4. Allow to cool below 45°C.
5. If desired, aseptically add 10 ml sterile 10% glucose solution and mix thoroughly.

Specimen Collection and Preparation

Not applicable

Test Procedure

Consult appropriate references for recommended test procedures.^{1,2,3}

Results

Growth is evident in the form of turbidity.

References

1. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**:190.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
3. **Miller, J. H.** 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Packaging

LB Broth, Lennox	500 g	0402-17
	2 kg	0402-07
	10 kg	0402-08

Bacto® LB Broth, Miller

Intended Use

Bacto LB Broth, Miller (Luria-Bertani) is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures.

Summary and Explanation

LB Broth, Miller is based on LB Medium as described by Miller for the growth and maintenance of *Escherichia coli* strains used in molecular microbiology procedures.^{1,3} LB Broth, Miller is a nutritionally rich medium designed for growth of pure cultures of recombinant strains. *Escherichia coli* is grown to late log phase in LB Medium. Some plasmid vectors replicate to a high copy number without selective amplification. Some vectors do not replicate so freely, and need to be selectively amplified. Chloramphenicol has been added to inhibit host synthesis and as a result, prevents replication of the bacterial chromosome.⁴

LB Broth, Miller contains twenty times the sodium chloride level of Luria Broth Base, Miller and twice the level found in LB Broth, Lennox.^{3,5} This allows the researcher to select the optimal salt concentration for a specific strain.

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by sodium chloride.

Formula

LB Broth, Miller

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	10 g
Final pH 7.0 ± 0.2 at 25°C	

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white to beige, free-flowing, homogeneous.
Solution:	2.5% solution; soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.
Prepared Tubes:	Very light amber, clear to very slightly opalescent.
Reaction of 2.5% Medium at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare LB Broth, Miller per label directions. Inoculate the tubes and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	33526	100-1,000	Good

The culture listed above is the minimum that should be used for performance testing.

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 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

LB Broth, Miller

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve 25 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.^{3,4}

Results

Growth should be evident by the appearance of turbidity in the medium.

References

1. **Luria, S. E., and J. W. Burrous.** 1955. Hybridization between *Escherichia coli* and *Shigella*. *J. Bacteriol.* **74**:461-476.
2. **Luria, S. E., J. N. Adams, and R. C. Ting.** 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology.* **12**:348-390.
3. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
4. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
5. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology.* **1**:190-206.

Packaging

LB Broth, Miller	500 g	0446-17
	2 kg	0446-07

Bacto® LPM Agar Base

Bacto Moxalactam Antimicrobial Supplement

Intended Use

LPM Agar Base is used with Bacto Moxalactam Antimicrobial Supplement for isolating and cultivating *Listeria monocytogenes*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,¹ *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.² The first reported food-borne outbreak of listeriosis was in 1985,³ and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.⁴

Implicated vehicles of transmission include turkey frankfurters,⁵ coleslaw, pasteurized milk, Mexican-style cheese, pâté, and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.⁶

Listeria species grow over a pH range of 5.0-9.6, and survive in food products with pH levels outside these parameters.⁷ *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.⁸

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

LPM Agar, a modification of McBride Listeria Agar, was developed by Lee and McClain⁹ to recover low numbers of *Listeria monocytogenes* from samples with profusely mixed microflora. Its use is recommended when testing food and dairy samples and clinical specimens for *Listeria*.

User Quality Control

Identity Specifications

LPM Agar Base

Dehydrated Appearance: Light tan, homogeneous, may have a tendency to form soft lumps.

Solution: 5.05% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent.

Prepared Plates: Light to medium amber, slightly opalescent.

Reaction of 5.05% Solution at 25°C: pH 7.3 ± 0.2

Moxalactam Antimicrobial Supplement

Lyophilized Appearance: White to off-white cake (may be broken).

Solution: Yellow tinted, clear solution when rehydrated with 10 ml sterile distilled or deionized water.

Cultural Response

Prepare LPM Agar Base with Moxalactam Antimicrobial Supplement. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

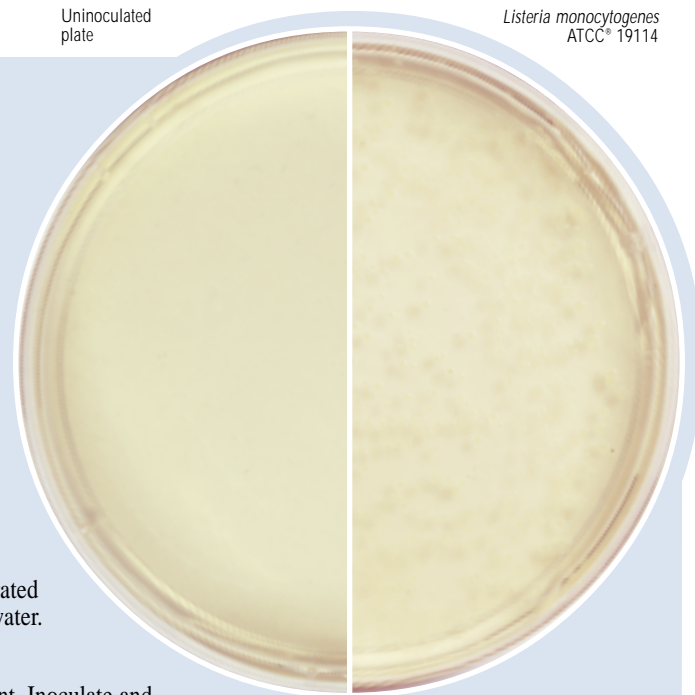
ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Enterococcus faecalis</i>	29212*	1,000-2,000	marked to complete inhibition
<i>Escherichia coli</i>	25922*	1,000-2,000	marked to complete inhibition
<i>Listeria monocytogenes</i>	19114	100-1,000	good at 40-48 hours

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 plate

Listeria monocytogenes
ATCC® 19114



Principles of the Procedure

In LPM Agar, Tryptose and Beef Extract provide nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. Glycine anhydride is used for improved recovery of *Listeria*. Lithium chloride, in an increased concentration, and phenylethanol are incorporated to aid in suppression of both gram-positive and gram-negative contaminants. Agar is a solidifying agent. Moxalactam Antimicrobial Supplement is added to LPM Agar Base after autoclaving to inhibit staphylococci, bacilli and *Proteus* species.

Formula

LPM Agar Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Sodium Chloride	5 g
Lithium Chloride	5 g
Glycine Anhydride	10 g
Phenylethanol	2.5 g
Bacto Agar	15 g
Final pH 7.3 ± 0.2 at 25°C	

Moxalactam Antimicrobial Supplement

Formula per 10 ml

Moxalactam	20 mg
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Precautions

1. For Laboratory Use.
2. **LPM Agar 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, Face, Muscles, Nerves, Urogenital.

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.

Moxalactam Antimicrobial Supplement:

MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Liver, Kidneys

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 procedure in handling and disposing of infectious materials.

Storage

1. Store LPM Agar Base at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.
Store Moxalactam Antimicrobial 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

LPM Agar Base
Moxalactam Antimicrobial Supplement

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 50.5 grams of LPM Agar Base in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45-50°C in a waterbath.
5. Aseptically add 10 ml Moxalactam Antimicrobial Supplement rehydrated per label instructions with sterile distilled or deionized water.
6. Mix well and dispense into Petri dishes.

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.^{7,10,11,12}
2. Clinical specimens obtained from nonsterile sites, foods, and specimens obtained from the environment should be selectively enriched for *Listeria* spp. before being plated.¹⁰
3. Process each specimen, using procedures appropriate for that specimen or sample.^{7,10,11,12}

Test Procedure

Clinical specimens obtained from nonsterile sites should be selectively enriched for *Listeria* spp. before being plated. Please refer to appropriate references for the procedure to use with clinical specimens.¹⁰ For a procedure for isolating *Listeria* from milk, milk products and food samples, refer to an appropriate reference.^{7,11,12}

Results

Observe colonies under oblique transmitted light. *Listeria* colonies display a grey to blue color with a ground glass appearance.

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.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low- and high-fat, frozen and refrigerated ground beef. *J. Food Prot.* **57**:969-974.
3. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma. Special Report. *J. Assoc. Off. Anal. Chem.* **70**:769-772.
4. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. *J. Food Prot.* **58**:604-608.
5. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. *J. Food Prot.* **55**:4-7.
6. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. *J. Food Prot.* **58**:244-250.
7. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*. 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.
8. **Kramer, P. A., and D. Jones.** 1969. Media selective for *Listeria monocytogenes*. *J. Appl. Bacteriol.* **32**:381-394.
9. **Lee, W. H., and D. McClain.** 1986. Improved *Listeria monocytogenes* selective agar. *Appl. Environ. Microbiol.* **52**:1215-1217.
10. **Swaminathan, B., J. Rocourt, and J. Bille.** 1995. *Listeria*, p. 342-343. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R.H. Tenover (eds.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
11. **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.
12. **Hitchins, A. D.** 1992. *Listeria monocytogenes*, p. 141-151. *FDA Bacteriological analytical manual*, 7th ed. AOAC International, Arlington, VA.

Packaging

LPM Agar Base	500 g	0221-17
	2 kg	0221-07
Moxalactam Antimicrobial Supplement	6 x 10 ml	0216-60

Bacto® Lactobacilli Agar AOAC

Bacto Lactobacilli Broth AOAC

Intended Use

Bacto Lactobacilli Agar AOAC is used for maintaining stock cultures used in the microbiological assays of vitamins and amino acids.

Bacto Lactobacilli Broth AOAC is used for preparing inocula used in the microbiological assays of vitamins and amino acids.

Summary and Explanation

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

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

Lactobacilli Agar AOAC¹ and Lactobacilli Broth AOAC¹ are prepared according to the formula recommended by Loy.² Lactobacilli Agar AOAC is used for maintaining stock cultures. Lactobacilli Broth AOAC is used to prepare inocula of *Lactobacillus leichmannii* ATCC® 7830, *Enterococcus faecium* ATCC 8043, *Lactobacillus plantarum*

ATCC 8014, *Lactobacillus casei* ATCC 7469 and other organisms used in the microbiological assay of B vitamins.

Lactobacillus species grow poorly on non-selective culture media and require special nutrients. Mickle and Breed³ reported the use of tomato juice in culture media for lactobacilli. Kulp,⁴ while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.

Principles of the Procedure

Peptonized Milk and Yeast Extract provides the nitrogen, amino acids and vitamins sources in Lactobacilli Agar AOAC and Lactobacilli Broth AOAC. Dextrose is a carbon source to facilitate organism growth. Tomato juice creates the proper acidic environment. Potassium Phosphate Monobasic is a buffering agent. Tween® 80 (Sorbitan Monooleate Complex) acts as an emulsifier. Bacto Agar is a solidifying agent in Lactobacilli Agar AOAC.

Formula

Lactobacilli Agar AOAC

Formula Per Liter	
Peptonized Milk	15 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Tomato Juice (100 ml)	5 g
Potassium Phosphate Monobasic	2 g
Sorbitan Monooleate Complex	1 g
Bacto Agar	10 g
Final pH 6.8 ± 0.2 at 25°C	

Lactobacilli Broth AOAC

Formula Per Liter

Peptonized Milk	5 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Tomato Juice (100 ml)	5 g
Monobasic Potassium Phosphate	2 g
Tween® 80	1 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.
3. Take care to avoid contamination of media or glassware used for microbiological assay procedures. Extremely small amounts of

foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemical must be used.

Storage

Store the dehydrated medium below 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**

Lactobacilli Agar AOAC
Lactobacilli Broth AOAC

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Inoculating needle
0.9% NaCl

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:
Lactobacilli Agar AOAC - 48 grams/liter
Lactobacilli Broth AOAC - 38 grams/liter
2. Heat to boiling 2-3 minutes to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Samples are prepared for assay according to references given in the specific assay procedures. The samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure**Stock Cultures**

1. Prepare stock cultures in one or more tubes of sterile Lactobacilli Agar AOAC.
2. Inoculate the medium using an inoculating needle.
3. Incubate at 30-37°C for 18-24 hours.
4. Store at 2-8°C.
5. Transfer at weekly or twice monthly intervals.

Inoculum

1. Subculture from a 16-24 hour stock culture into 10 ml Lactobacilli Broth AOAC.
2. Incubate at 35-37°C for 16-24 hours or as specified in specific assay procedures.
3. Centrifuge the culture and decant the supernatant.
4. Resuspend cells in 10 ml of sterile 0.9% NaCl solution or sterile single-strength basal assay medium.

User Quality Control**Identity Specifications****Lactobacilli Agar AOAC**

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 4.8% solution, soluble in distilled or deionized water on boiling 2-3 minutes.

Prepared Medium: Medium amber, opalescent when hot, clearer when cooled to 45-50°C.

Reaction of 4.8%

Solution at 25°C: pH 6.8 ± 0.2

Lactobacilli Broth AOAC

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 3.8% solution, soluble in distilled or deionized water on boiling 2-3 minutes. Medium amber, clear, may have a slight precipitate.

Prepared Medium: Medium amber, opalescent when hot, clear with a very slight precipitate when cooled.

Reaction of 3.8%

Solution at 25°C: pH 6.8 ± 0.2

Cultural Response

Prepare Lactobacilli Agar AOAC and Lactobacilli Broth AOAC per label directions. Inoculate Lactobacilli Agar AOAC by stabbing the medium with test organisms; incubate at 35 ± 2°C for 18-48 hours. Inoculate Lactobacilli Broth AOAC with test organisms and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Enterococcus hirae</i>	8043	100-1,000	good
<i>Lactobacillus casei</i> subsp. <i>rhamnosus</i>	7469	100-1,000	good
<i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i>	7830	100-1,000	good
<i>Lactobacillus plantarum</i>	8014	100-1,000	good

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

- Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise indicated.
- Dilute the washed suspension 1:100 with sterile 0.9% NaCl or sterile single-strength basal assay medium or as indicated. Where applicable, inoculum concentration should be adjusted according to limits specified in AOAC¹ or US Pharmacopeia.⁵

For a complete discussion on vitamin assay methodology refer to appropriate procedures outlined in the references.^{1,5}

Results

Refer to appropriate references for vitamin assay results.^{1,5}

Limitations of the Procedure

- The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for that purpose.
- Aseptic technique should be used throughout the vitamin assay procedure.
- The use of altered or deficient media may result in mutants with different nutritional requirements that will not give a satisfactory response.

- For a successful completion of these procedures, all conditions of the assay must be adhered to meticulously.

References

- Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
- Loy.** 1958. J. AOAC. 4:61.
- Mickle and Breed.** 1925. Technical Bulletin 110, NY State Agriculture Ex. Station.
- Kulp, J. W. L., and V. White.** 1932. Modified medium for plating *Lactobacillus acidophilus*. Science 76:17.
- The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc., Rockville, MD.

Packaging

Lactobacilli Agar AOAC	100 g	0900-15*
Lactobacilli Broth AOAC	100 g	0901-15*

*Store at 2-8°C

Bacto® Lactobacilli MRS Agar Bacto Lactobacilli MRS Broth

Intended Use

Bacto Lactobacilli MRS Agar and Bacto Lactobacilli MRS Broth are recommended for use in the isolation, enumeration and cultivation of *Lactobacillus* species.

Also Known As

“MRS” is an abbreviation for the authors’ names, deMan, Rogosa and Sharpe.

Summary and Explanation

Lactobacilli MRS Agar and Lactobacilli MRS Broth are based on the formulations of deMan, Rogosa and Sharpe.¹ These media were shown by the authors to support luxuriant growth of all lactobacilli from oral, fecal, dairy and other sources.

Principles of the Procedure

Lactobacilli MRS Agar and Lactobacilli MRS Broth contain Peptone and Dextrose. These ingredients supply nitrogen, carbon and other elements necessary for growth. Polysorbate 80, Acetate, Magnesium and Manganese provide growth factors for culturing a variety of lactobacilli. The above ingredients may inhibit the growth of some organisms other than lactobacilli.

Formula

Lactobacilli MRS Agar

Formula Per Liter	
Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	10 g

Bacto Yeast Extract	5 g
Dextrose	20 g
Sorbitan Monooleate Complex	1 g
Ammonium Citrate	2 g
Sodium Acetate	5 g
Magnesium Sulfate	0.1 g
Manganese Sulfate	0.05 g
Potassium Phosphate, Dibasic	2 g
Bacto Agar	15 g
Final pH 6.5 ± 0.2 at 25°C	

Lactobacilli MRS Broth

Formula Per Liter	
Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	10 g
Bacto Yeast Extract	5 g
Dextrose	20 g
Sorbitan Monooleate Complex	1 g
Ammonium Citrate	2 g
Sodium Acetate	5 g
Magnesium Sulfate	0.1 g
Manganese Sulfate	0.05 g
Potassium Phosphate, Dibasic	2 g
Final pH 6.5 ± 0.2 at 25°C	

Precautions

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

Storage

Store Lactobacilli MRS Agar and Lactobacilli MRS Broth at 2-8°C. The powders are very hygroscopic. Keep containers tightly closed. 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

Lactobacilli MRS Agar
Lactobacilli MRS Broth

Materials Required But Not Provided

Flasks with closures
Sterile Petri dishes
Distilled or deionized water
Autoclave
Waterbath (45-50°C)
Incubator (30 or 35°C)

Method of Preparation

Lactobacilli MRS Agar

1. Suspend 70 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Lactobacilli MRS Broth

1. Suspend 55 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

1. Collect samples in sterile containers and transport immediately to the laboratory following recommended guidelines.^{2,3,4}
2. Process each sample using procedures appropriate for that sample.^{2,3,4}

Test Procedure

Direct Counts

1. To obtain direct counts of lactobacilli, pour 15-20 ml sterile, molten (45-50°C) Lactobacilli MRS Agar into sterile Petri dishes containing 1 ml volumes of diluted test sample.
2. Distribute the inoculum throughout the medium by rotating the plate in one direction and then in the reverse direction.
3. Allow the medium to solidify on a flat surface for 5-10 minutes.
4. Alternatively, plates of Lactobacilli MRS Agar can be used for direct recovery of organisms using the streak inoculation technique.
5. Incubate agar plates at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere supplemented with carbon dioxide.

Broth Enrichment

1. Samples can be inoculated directly into Lactobacilli MRS Broth.
2. Incubate broth tubes at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere.
3. Subculture growth in broth tubes to appropriate solid media.

User Quality Control

Identity Specifications

Lactobacilli MRS Agar

Dehydrated Appearance: Light tan, free-flowing, homogeneous.

Solution: 7.0% solution, soluble in distilled or deionized water upon boiling. Solution is dark amber, clear to slightly opalescent.

Prepared Medium: Medium amber, very slightly to slightly opalescent.

Reaction of 7.0%
Solution at 25°C: pH 6.5 ± 0.2

Lactobacilli MRS Broth

Dehydrated Appearance: Tan, free-flowing, homogeneous.

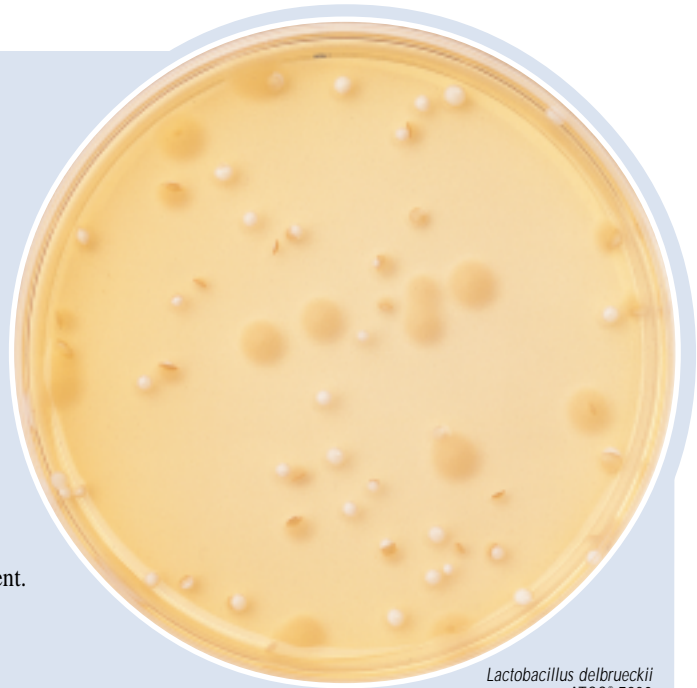
Solution: 5.5% solution, soluble in distilled or deionized water upon boiling. Solution is dark amber, clear to very slightly opalescent.

Reaction of 5.5%
Solution at 25°C: pH 6.5 ± 0.2

Cultural Response

Prepare Lactobacilli MRS Agar or Lactobacilli MRS Broth per label directions. Inoculate Lactobacilli MRS Agar and incubate in a 5% CO₂ atmosphere at 35°C for 24- 72 hours. Inoculate Lactobacilli MRS Broth and incubate at 35°C for 24 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	7830	100-1,000	good
<i>Lactobacillus fermentum</i>	9338	100-1,000	good
<i>Lactobacillus</i> species	11506	100-1,000	good



Lactobacillus delbrueckii
ATCC® 7830

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

Lactobacilli appear as large, white colonies embedded in or on Lactobacilli MRS Agar or as turbidity in Lactobacilli MRS Broth. Growth may be subcultured onto the appropriate media for use in additional procedures. Refer to appropriate references for recommendations on the culture of *Lactobacillus* spp.^{2,3,4}

Limitations of the Procedure

- Organisms other than lactobacilli may grow in these media. Isolates must be confirmed as lactobacilli by appropriate biochemical testing.

References

- deMan, J. C., M. Rogosa, and M. E. Sharpe.** 1960. A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* **23**:130.

- Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
- MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
- Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Lactobacilli MRS Agar	500 g	0882-17
Lactobacilli MRS Broth	500 g	0881-17
	2 kg	0881-07
	10 kg	0881-08

Bacto® Lactose Broth

Intended Use

Bacto Lactose Broth is used for cultivating *Salmonella* and coliform organisms in water, foods, dairy and pharmaceutical products.

Summary and Explanation

Lactose Broth is frequently used as a pre-enrichment medium when testing foods and dairy products for *Salmonella*. In dried or processed foods, salmonellae may be sublethally injured and in low numbers. The presence of other bacteria as well as components of the food sample may hinder growth and recovery of *Salmonella*. Pre-enrichment in a nonselective medium such as Lactose Broth allows for repair of cell damage, dilutes toxic or inhibitory substances, and provides a nutritional

advantage to *Salmonella* over other bacteria.¹ Lactose Broth is widely used and is included in many Standard Methods procedures for testing foods, dairy products and other materials.^{1,2,3,4}

In past years, Lactose Broth was recommended for detection of coliforms in water⁵ and foods⁶ and for the confirmed phase in testing dairy products.⁷

Principles of the Procedure

Lactose Broth contains Beef Extract and Peptone as carbon and nitrogen sources for general growth requirements. Lactose is a carbohydrate source.

The purpose of a pre-enrichment medium is to provide a higher ratio of *Salmonella* to non-*Salmonella* bacteria after incubation. Most

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to light tan, free-flowing, homogeneous.
Solution:	1.3% solution, soluble in distilled or deionized water with slight warming. Light to medium amber, clear without significant precipitate.
Prepared Medium:	Light to medium amber, clear without significant precipitate.
Reaction of 1.3% Solution at 25°C:	6.9 ± 0.2

Cultural Response

Prepare Lactose Broth (dehydrated) per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH	GAS	ACID
<i>Escherichia coli</i>	25922*	100-1,000	good	+	+
<i>Salmonella typhi</i>	6539	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.



Uninoculated tube

Escherichia coli ATCC® 25922

non-*Salmonella* bacteria ferment lactose while *Salmonella* does not. As lactose-fermenting bacteria metabolize lactose, the pH of the medium decreases, creating a bacteriostatic effect on competing microorganisms.

Formula

Bacto Lactose Broth

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Lactose	5 g
Final pH 6.9 ± 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.

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

Lactose Broth

Materials Required but not Provided

Flasks with closures
 Distilled or deionized water
 Autoclave
 Incubator (35°C)
 Tubes with closures
 Fermentation vials
 Disinfectant solution (for prepared Lactose Broth)

Method of Preparation

Lactose Broth (dehydrated)

1. Suspend 13 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

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Lactose Broth is used in the pre-enrichment phase of the preparation

of food samples for isolation of *Salmonella*. Consult standard references for specific instructions for each type of material being tested.^{1,2,3,4}

1. Transfer a 25 gram or 25 ml sample of test material into a container. Add 225 ml of sterile Lactose Broth. Mix as necessary to make a homogeneous suspension. Incubate at 35°C for 24 ± 2 hours.
2. Transfer 1 ml of suspension to appropriate enrichment broths, such as Tetrathionate Broth and Selenite Cystine Broth. Incubate at 35°C for 24 ± 2 hours.
3. Transfer a loopful of suspension to appropriate selective agar media, such as Hektoen Enteric Agar, XLD Agar and Bismuth Sulfite Agar. Incubate at 35°C for 24 ± 2 hours.

Results

Pre-enrichment, selective enrichment and selective plating increase the likelihood of isolating *Salmonella* from foods and other materials.

References

1. **Flowers, R. S., J. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-442. 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.
2. **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.
3. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. In Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Andrews, W. H.** 1995. Microbial Methods, p. 1-119. In Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
5. **American Public Health Association.** 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
6. **American Public Health Association.** 1976. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
7. **American Public Health Association.** 1978. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.

Packaging

Lactose Broth	100 g	0004-15
	500 g	0004-17
	2 kg	0004-07
	10 kg	0004-07
Lactose Broth	10 x 90 ml	9070-73

Bacto® Lactose Peptone Broth

Intended Use

Bacto Lactose Peptone Broth is used for the detection of coliform organisms in water.

Summary and Explanation

Lactose Peptone Broth is based on the Lactose Peptone Broth formula described in German Standard Methods and German Drinking Water Regulations.¹ Lactose Peptone Broth is recommended as a non-selective broth enrichment and detection medium for *E. coli* and other coliform bacteria present in water. Lactose fermentation and gas production at $36 \pm 1^\circ\text{C}$ are used as the basis for this presumptive coliform test.

Principles of the Procedure

Lactose Peptone Broth contains Tryptone and Soytone which provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Lactose is provided as a source of fermentable carbohydrate. Sodium Chloride is present in the medium to provide a suitable osmotic environment. Brom Cresol Purple is used as a colorimetric indicator to show the production of acid from the fermentation of lactose.

Formula

Lactose Peptone Broth

Formula Per Liter

Bacto Tryptone 17 g
Bacto Soytone 3 g

Bacto Lactose 10 g
Sodium Chloride 5 g
Brom Cresol Purple 0.02 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. Store prepared medium at $2-8^\circ\text{C}$.

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

Lactose Peptone Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Durham tubes
Autoclave
Incubator ($36 \pm 1^\circ\text{C}$)

Specimen Collection and Preparation

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

Method of Preparation

1. To prepare a triple strength solution, dissolve 105 grams in 1 liter of distilled or deionized water. To prepare a single strength solution, dissolve 35 grams in 1 liter of distilled or deionized water.
2. Dispense 50 ml into tubes or bottles containing a Durham tube.
3. Autoclave at 121°C for 15 minutes.

Test Procedure¹

Direct Broth Method

1. Add 100 ml of sample to 50 ml of triple strength Lactose Peptone Broth.
2. Incubate at $36 \pm 1^\circ\text{C}$ for 24-48 hours.
3. Examine tubes or bottles for evidence of acid formation and gas production.

Membrane Filtration Broth Method

1. Filter 100 ml of sample through a sterile 0.45 micron membrane filter.
2. Remove filter and place in 50 ml of single strength Lactose Peptone Broth.
3. Incubate at $36 \pm 1^\circ\text{C}$ for 24-48 hours.
4. Examine tubes or bottles for evidence of acid formation and gas production.

User Quality Control

Identity Specifications

Dehydrated Medium: Light beige, free-flowing, homogeneous.

Solution: 10.5% solution (triple-strength), soluble in distilled or deionized water. Solution is dark reddish-purple, clear to slightly opalescent.

Prepared Tubes: Dark reddish purple, clear to slightly opalescent.

Reaction of 10.5% Solution at 25°C : pH 7.4 ± 0.2

Cultural Response

Prepare Lactose Peptone Broth per label directions. Inoculate and incubate at $35 \pm 2^\circ\text{C}$ for 24-48 hours.

ORGANISM	ATCC*	INOCULUM CFU	LACTOSE FERMENTATION	GAS PRODUCTION
<i>Escherichia coli</i>	25922*	10-100	+	+
<i>Salmonella typhimurium</i>	14028*	100-1,000	-	-

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.

coliforms. Potassium Phosphates are the buffering agents, and Sodium Chloride is used to maintain the osmotic balance of the medium. Sodium Lauryl Sulfate is the selective agent used to inhibit organisms other than coliforms.

Formula

Lauryl Tryptose Broth

Formula Per Liter

Bacto Tryptose	20 g
Bacto Lactose	5 g
Potassium Phosphate, Dibasic	2.75 g
Potassium Phosphate, Monobasic	2.75 g
Sodium Chloride	5 g
Sodium Lauryl Sulfate	0.1 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.

NOTE: Refrigerated Lauryl Tryptose Broth generally becomes cloudy or forms precipitates. Incubate medium overnight at room temperature (20°C) before use to clear the medium.⁵

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

Lauryl Tryptose Broth

Materials Required But Not Provided

Glassware
 Test tubes
 Incubator (35°C)
 Fermentation vials
 Autoclave
 Distilled or deionized water

Method of Preparation

1. Suspend 35.6 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.

NOTE: Lauryl Tryptose Broth may be prepared in single strength when examining 1 ml or less of water as an inoculum. For inocula of 10 ml consult the table below.

PREPARATION OF LAURYL TRYPTOSE BROTH⁵

Inoculum mL	Amount of Medium in Tube mL	Volume of Medium+Inoculum mL	Dehydrated Lauryl Tryptose Broth Required g/L
1	10 or more	11 or more	35.6
10	10	20	71.2
10	20	30	53.4
20	10	30	106.8
100	50	150	106.8
100	35	135	137.1
100	20	120	213.6

Specimen Collection and Preparation

Collect and process specimens according to laboratory policy or standard methods.^{1,5,6,7,8,9}

Test Procedure

Follow the methods and procedures for the detection of coliform organisms as described in standard methods.^{1,5,6,7,8,9}

Results

After incubation of the tubes at 35 ± 2°C for 24 hours, examine for turbidity and gas production. If no gas has formed in the inverted tube, reincubate and reexamine after 48 hours.^{5,6}

Turbidity of the medium accompanied by formation of gas within 48 hours is a positive presumptive test for the presence of coliforms.^{5,6} The result should be confirmed by additional standard testing.^{1,5,6,7,8,9}

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. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1992. Coliform and other indicator bacteria, p. 247-267. *In* R. T. Marshal (ed.). Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
2. **Mallmann, W. L., and C. W. Darby.** 1941. Uses of a lauryl sulphate tryptose broth for the detection of coliform organisms. *Am. J. Public Health.* **31**:127.
3. **Darby, C. W., and W. L. Mallmann.** 1939. *J. of Am. Water Works Assoc.* **31**:689.
4. **Perry and Hajna.** 1944. *Am. J. Public Health.* **34**:735.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

8. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
9. **Bordner, R., and J. Winter (ed.).** 1978. Microbiological methods for monitoring the environment; water and wastes. Environmental monitoring and support laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Packaging

Lauryl Tryptose Broth	100 g	0241-15
	500 g	0241-17
	2 kg	0241-07
	10 kg	0241-08

Leptospira Medium EMJH

Bacto® Leptospira Medium Base EMJH · Bacto Leptospira Enrichment EMJH

Intended Use

Bacto Leptospira Medium Base EMJH is used with Bacto Leptospira Enrichment EMJH in cultivating *Leptospira*.

Summary and Explanation

In 1816, Adolf Weil described the first recognized leptospiral infections in humans.¹ These cases were caused by *Leptospira icterohaemorrhagiae* and the disease was subsequently named Weil's Disease.¹ Leptospirosis is a zoonotic disease, having its reservoir in wild, domestic, and peridomestic animals. Infection usually results from direct or indirect exposure to the urine of leptospiruric animals.²

Indirect exposure through contaminated water and soil accounts for most sporadic cases. Direct exposure occurs in pet owners, veterinarians and persons working with livestock.³

The basal medium and enrichment are prepared according to the formulations described by Ellinghausen and McCullough⁴ as modified by Johnson and Harris.⁵ They modified the formula by replacing rabbit serum medium with Tween 80-albumin. Leptospira Medium EMJH was used in cultivation studies of *Leptospira*.⁶

Leptospira Medium EMJH is recommended for the clinical isolation of *Leptospira*.^{7,8}

User Quality Control

Identity Specifications

Leptospira Medium Base EMJH

Dehydrated Appearance: White, free-flowing, homogeneous.

Basal Solution: 2.3 grams of base in 900 ml distilled or deionized water; soluble upon agitation; colorless, clear with no significant precipitate.

Prepared Medium w/Enrichment: Very light to light amber, clear with no precipitate.

Reaction (Basal Medium) at 25°C: pH 7.5 ± 0.2

Leptospira Enrichment EMJH

Appearance: Medium to dark amber, clear to very slightly opalescent with no significant precipitate.

Cultural Response

Prepare the complete Leptospira Medium EMJH per label directions. Inoculate tubes with undiluted *Leptospira* and incubate at 30 ± 2°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
<i>Leptospira interrogans</i> serovar <i>australis</i>	23605	one drop	good
<i>Leptospira interrogans</i> serovar <i>canicola</i>	23470	one drop	good
<i>Leptospira interrogans</i> serovar <i>grippityphosa</i>	23604	one drop	good

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



Uninoculated tube

Leptospira interrogans ATCC® 23605

Principles of the Procedure

Leptospira Medium Base EMJH contains ammonium chloride, a nitrogen source, and thiamine, a growth factor. Sodium phosphate dibasic and potassium phosphate monobasic are buffering agents. Sodium chloride maintains the osmotic balance of this formula.

Leptospira Enrichment EMJH contains albumin, polysorbate 80 and additional growth factors for *Leptospira*.

Formula

Leptospira Medium Base EMJH

Formula Per Liter

Sodium Phosphate Dibasic	1 g
Potassium Phosphate Monobasic	0.3 g
Sodium Chloride	1 g
Ammonium Chloride	0.25 g
Thiamine	0.005 g
Final pH 7.5 ± 0.2 at 25°C	

Leptospira Enrichment EMJH

A solution of albumin, polysorbate 80 and additional growth factors for *Leptospira*.

Precautions

- For Laboratory Use.
- Leptospira Medium Base EMJH:**
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.
- Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

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

Store Leptospira Enrichment EMJH 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

Leptospira Medium Base EMJH or
Leptospira Enrichment EMJH

Materials Required But Not Provided

Glassware
Autoclave
Incubator (30°C) (optional)
Sterile tubes

Method of Preparation

- Dissolve 2.3 grams Leptospira Medium EMJH in 900 ml distilled or deionized water.
- Autoclave at 121°C for 15 minutes.
- Aseptically add 100 ml Leptospira Enrichment EMJH to the basal medium at room temperature. Mix thoroughly.

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.⁵

Test Procedure

Culture Procedures⁷

Blood and Spinal Fluid

Freshly drawn blood is preferable; otherwise, use blood taken with SPS, sodium oxalate or heparin.

- Inoculate four 5 ml tubes of Leptospira Medium EMJH with 1-2 drops of fluid per tube.
- Incubate in the dark at 28-30°C or at room temperature.

Urine

A total of 12 tubes will be inoculated for each urine specimen.

- Prepare 1:10 and 1:100 dilutions of urine using Leptospira Medium EMJH to dilute potential inhibitory substances.
- Inoculate two 5 ml tubes each of Leptospira Medium EMJH with:
Urine undiluted, 1 drop per tube;
Urine diluted 1:10, 1 drop per tube;
Urine diluted 1:100, 1 drop per tube.
- Duplicate the above inoculations using medium containing 200 µg/ml 5- fluorouracil to inhibit contaminants.
- Incubate the tubes in the dark at 28-30°C or at room temperature.

Results⁷

- Examine tubes weekly for signs of growth (turbidity, haze, or a ring of growth).
- Examine tubes microscopically each week. Take a small drop from a few millimeters below the surface, and examine it with dark-field illumination. Use 400X magnification.
- Leptospires will be seen as tightly coiled spirochetes about 1 µm wide and 6-20 µm long. Leptospires rotate rapidly on their long axes and usually have hooked ends.
- If the specimen is positive, subculture about 0.5 ml taken from the area of growth to two tubes of fresh medium.

Limitations of the Procedure

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

References

- Elliott, S. H.** 1980. Discussion and clinical diagnosis of Leptospirosis. *J. Am. Med. Tech.* **42**:37-44.

- Faine, S. (ed.). 1982. Guidelines for the control of leptospirosis. W. H. O. Offset publication no. 67. World Health Organization, Geneva.
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- Ellinghausen, Jr., H. C., and W. G. McCullough. 1965. Nutrition of *Leptospira pomona* and growth of 13 other serotypes: fractionation of oleic albumin complex (OAC) and a medium of bovine albumin and polysorbate 80. Am. J. Vet. Research 26:45-51.
- Johnson, R., and V. G. Harris. 1967. Differentiation of pathogenic of leptospire. J. Bacteriol. 94:27-31.
- Rule, P. L., and A. D. Alexander. 1986. Gellan gum as a substitute for agar in leptospiral media. J. Clin. Microbiol. 23:500-504.
- Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
- 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.

Packaging

Leptospira Medium Base EMJH	500 g	0794-17
Leptospira Enrichment EMJH	6 x 100 ml	0795-73*

*Store at 2-8°C

Bacto® Lethen Agar Bacto Lethen Broth

Intended Use

Bacto Lethen Agar is used for evaluating the bactericidal activity of quaternary ammonium compounds.

Bacto Lethen Broth is used for determining the phenol coefficient of cationic surface-active materials.

Also Known As

“AOAC Lethen Agar/Broth” and “Trypticase Glucose Extract Agar with Lecithin and Tween 80” are common terms for Lethen Agar/Broth.

Summary and Explanation

The value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers was described by Weber and Black¹ in 1948. The addition of lecithin and Tween[®] 80 to Tryptone Glucose Extract (TGE) agar resulted in a medium that effectively neutralizes quaternary ammonium compounds in the testing of germicidal activity. Lethen Agar is a modification of TGE agar with the addition of lecithin and sorbitan monooleate (Tween 80).

Lethen Broth was developed as a subculture medium for the neutralization of quaternary ammonium compounds in disinfectant testing. Quisno, Gibby and Foter,² found that the addition of lecithin and Tween 80 to F.D.A. Broth resulted in a medium that neutralized high concentrations of quaternary ammonium salts. The resulting medium, termed “Lethen” (a combination of Lecithin and Tween) was easy to prepare and clear in appearance which aided in visual inspection for growth. Lethen Broth is recommended by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC)³ for use with disinfectants containing cationic surface active materials.

Lethen Agar and Lethen Broth are specified for use by the American Society for Testing Materials (ASTM) in the Standard Test Method for Preservatives in Water Containing Cosmetics.⁴

Principles of the Procedure

Lethen Agar contains Beef Extract and Tryptone which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Dextrose is provided as a source of fermentable carbohydrate. Bacto Agar is added as a solidifying agent. Lecithin and Sorbitan Monooleate are added to neutralize surface disinfectants.^{2,5,6} Lecithin is added to neutralize quaternary ammonium compounds and Sorbitan Monooleate is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol.⁷

Lethen Broth contains Peptamin and Beef Extract which provide the carbon and nitrogen sources necessary for growth. Lecithin and Tween 80 are added as surface active disinfectant neutralizing agents.^{2,5,6} Sodium Chloride is included to maintain osmotic balance.

Formula

Lethen Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Tryptone	5 g
Bacto Dextrose	1 g
Bacto Agar	15 g
Sorbitan Monooleate	7 g
Lecithin	1 g
Final pH 7.0 ± 0.2 at 25°C	

Lethen Broth

Formula Per Liter

Bacto Peptamin	10 g
Bacto Beef Extract	5 g
Lecithin	0.7 g
Tween 80	5 g
Sodium Chloride	5 g
Final pH 7.0 ± 0.2 at 25°C	

Precautions

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

Storage

Store the dehydrated media at 2-8°C. The dehydrated media are very hygroscopic. Keep containers 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

Lethen Agar

Dehydrated Appearance:	Tan, moist appearance, with a tendency to clump.
Solution:	3.2% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, clear to slightly opalescent, may have a slight, fine precipitate.
Prepared Plates:	Light to medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 3.2% Solution at 25°C:	pH 7.0 ± 0.2

Lethen Broth

Dehydrated Appearance:	Tan, moist appearance, with a tendency to clump.
Solution:	2.57% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, clear to slightly opalescent (opalescent when hot), May have a very slight precipitate.
Prepared Tubes:	Light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 2.57% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Lethen Agar per label directions. Using the pour plate technique, inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	11229	100-1,000	good
<i>Staphylococcus aureus</i>	6538	100-1,000	good

Prepare Lethen Broth per label directions. Inoculate and incubate at 35 ± 2°C for 40- 48 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	11229	100-1,000	good
<i>Staphylococcus aureus</i>	6538	100-1,000	good
<i>Salmonella typhi</i>	6539	100-1,000	good

Procedure

Materials Provided

Lethen Agar
Lethen Broth

Materials Required But Not Provided

Glassware
Distilled or deionized water|
Autoclave

Method of Preparation

1. **Lethen Agar:** Suspend 32 grams in 1 liter distilled or deionized water. Boil to dissolve completely.
Lethen Broth: Suspend 25.7 grams in 1 liter distilled or deionized water. Boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedures

Lethen Agar and Lethen Broth are used in a variety of procedures. Please consult appropriate references for further information.^{3,4}

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. The dehydrated Lethen Agar has a characteristic “brown sugar” appearance. This does not indicate deterioration.

References

1. **Weber, G. R., and L. A. Black.** 1948. Relative efficiency of quaternary inhibitors. *Soap and Sanit. Chem.* **24**:134-139.
2. **Quisno, R., I. W. Gibby, and M. J. Foter.** 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. *Am. J. Pharm.* **118**:320-323.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, D.C.
4. **American Society for Testing Materials.** 1991. Standard test method for preservatives in water-containing cosmetics, E 640-78. Annual Book of ASTM Standards, Philadelphia, PA.
5. **Erlanson, A. L., Jr., and C. A. Lawrence.** 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. *Science* **118**:274-276.
6. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. *App. Environ. Microbiol.* **32**:80-84.
7. **Favero (chm.).** 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Packaging

Lethen Agar	500 g	0680-17
Lethen Broth	500 g	0681-17

Bacto® Levine EMB Agar

Intended Use

Bacto Levine EMB Agar is used for isolating and differentiating lactose-fermenting from lactose-nonfermenting gram-negative enteric bacilli.

Summary and Explanation

Eosin methylene blue agar (EMB) was originally formulated by Holt-Harris and Teague.¹ The formulation contained eosin and methylene blue as inhibitors and pH indicators, and the carbohydrates lactose and sucrose. Levine^{2,3} modified the formulation by using lactose at an increased concentration and omitting sucrose.

Levine EMB agar is used for detection and confirmation of coliforms, specifically enteropathogenic *E. coli* in foods, dairy products and other materials.^{4,5,6,7}

Principles of the Procedure

Levine EMB Agar contains Bacto Peptone as a source of carbon and nitrogen for general growth requirements and Lactose as the carbohydrate. Eosin Y and Methylene Blue are pH indicators, as well as inhibitors of microorganisms other than gram-negative bacilli. However, some staphylococci, streptococci and yeast may grow as small pinpoint colonies. Bacto Agar is the solidifying agent.

Formula

Levine EMB Agar

Formula Per Liter	
Bacto Peptone	10 g
Bacto Lactose	10 g

Dipotassium Phosphate	2 g
Bacto Agar	15 g
Eosin Y	0.4 g
Methylene Blue.....	0.065 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 material.

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

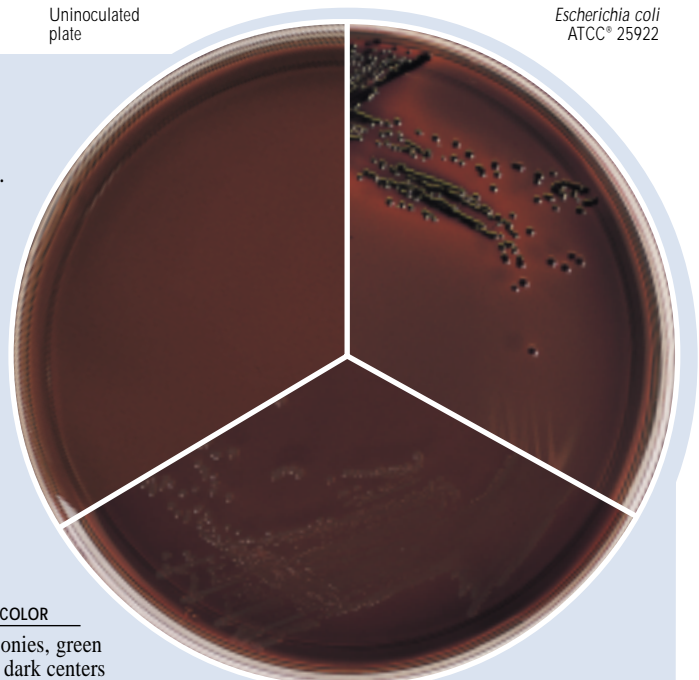
Levine EMB Agar

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Incubator (35°C)
Petri dishes

Uninoculated plate

Escherichia coli
ATCC® 25922



Salmonella typhimurium
ATCC® 14028

User Quality Control

Identity Specifications

Dehydrated Appearance: Reddish-pink free-flowing, homogeneous.

Solution: 3.75% solution soluble in distilled or deionized water on boiling; green to wine red color with an orange cast, slightly opalescent to opalescent. May have a flocculent precipitate.

Prepared medium: Wine red color with a green to orange cast, slightly opalescent with a finely dispersed flocculent precipitate.

Reaction of 3.75% solution at 25°C: pH 7.1 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
<i>Escherichia coli</i>	25922*	100-1,000	good	blue-black colonies, green metallic sheen dark centers
<i>Salmonella typhimurium</i>	14028*	100-1,000	good	colorless to amber
<i>Enterococcus faecalis</i>	29212*	1,000-2,000	partially	colorless 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.

Method of Preparation

1. Suspend 37.5 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. Evenly disperse the precipitate when dispensing.

Specimen Collection and Preparation

Consult standard references for specific instructions for the type of material being tested.^{4,5,6,7}

Test Procedure

Consult standard references for specific instructions for the type of material being tested.^{4,5,6,7}

Results

E. coli colonies are typically dark-centered with or without a metallic sheen.

Lactose fermenters:	Blue-black to brownish colored colonies, may have dark centers with or without metallic sheen.
Lactose non-fermenters:	Colorless or transparent, amber to light-purple colonies.
Staphylococci and streptococci:	Colorless pinpoint colonies.
Yeasts:	Colorless, dull pinpoint colonies; may be “spidery” at edges.

Limitations of the Procedure

1. Levine EMB Agar is only moderately inhibitory. Some staphylococci, streptococci, and yeasts may grow as small pinpoint colonies. Perform microscopic examination and biochemical tests to identify to genus and species.

2. The medium will support growth of most gram-negative bacilli but some strains of *Salmonella* and *Shigella* may be inhibited.

References

1. **Holt-Harris, J. E., and O. Teague.** 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools. *J. Infect. Dis.* **18**:596.
2. **Levine, M.** 1918. Differentiation of *E. coli* and *A. aerogenes* on a simplified eosin-methylene blue agar. *J. Infect. Dis.* **23**:43-47.
3. **Levine, M.** 1921. Bacteria fermenting lactose—the significance in water analysis. *Bull. 62. Iowa State College Eng. Exp. Sta., Ames, Iowa.*
4. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms - *Escherichia coli* and its toxins, p. 325-369. *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.
5. **Marshall, R. T. (ed.).** 1993. *Standard methods for the microbiological examination of dairy products*, 16th ed. American Public Health Association, Washington, D.C.
6. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler.** 1995. *Escherichia coli* and the coliform bacteria. p. 4.01-4.29. *In* *Bacteriological analytical manual*, 8th ed. AOAC International, Gaithersburg, MD.
7. **Andrews, W.** 1995. *Microbial Methods*, p. 1-119. *In* *Official methods of analysis of AOAC International*, 16th ed. AOAC International, Arlington, VA.

Packaging

Levine EMB Agar	100 g	0005-15
	500 g	0005-17
	2 kg	0005-07
	10 kg	0005-08

Bacto® Lima Bean Agar

Intended Use

Bacto Lima Bean Agar is used for cultivating fungi.

Summary and Explanation

Fungi are ubiquitous in nature.¹ Of the estimated 250,000 species, fewer than 150 are known to be primary human pathogens.¹

Lima Bean Agar is prepared from an infusion of dry lima beans and is solidified with 1.5% agar. The nutritive properties of lima beans and the low pH of Lima Bean Agar create a suitable environment for the growth of many fungi.

Principles of the Procedure

Infusion from Lima Beans is a source of nitrogen, carbon, amino acids and vitamins. Bacto Agar is a solidifying agent.

User Quality Control**Identity Specifications**

Dehydrated Appearance:	Light yellowish tan, free-flowing, homogeneous.
Solution:	2.3% solution; soluble in distilled or deionized water on boiling. Solution is light to medium amber, opalescent, may have a slight precipitate.
Prepared Media:	Light to medium amber, opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Prepare Lima Bean Agar per label directions. Inoculate and incubate at 30 ± 2° C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
<i>Aspergillus niger</i>	16404	100-1,000	good
<i>Saccharomyces cerevisiae</i>	9763	100-1,000	good
<i>Candida albicans</i>	10231	100-1,000	good

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

Formula

Lima Bean Agar

Formula Per Liter	
Lima Bean, Infusion from	62.5 g
Bacto Agar	15 g
Final pH	5.6 ± 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

Lima Bean Agar

Materials Required but not Provided

Glassware
Autoclave

Sterile Petri dishes
Waterbath

Method of Preparation

1. Suspend 23 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

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

Results

Refer to appropriate references and procedures.

References

1. **Dixon, D. M., and R. A. Fromtling.** 1995. Morphology, taxonomy, and classification of the fungi, p. 699-708. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Lima Bean Agar	500 g	0117-17
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Bacto® Listeria Enrichment Broth

Intended Use

Bacto Listeria Enrichment Broth is used to selectively enrich *Listeria* from food.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,¹ *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.² The first reported food-borne outbreak of listeriosis was in 1985,³ and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.⁴

Implicated vehicles of transmission include turkey frankfurters,⁵ coleslaw, pasteurized milk, Mexican-style cheese, paté, and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.⁶

Listeria species grow over a pH range of 5.0-9.6, and survive in food products with pH levels outside these parameters.⁷ *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.⁸

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Listeria Enrichment Broth is based on the formula developed by Lovett *et al.*⁹ in which Tryptic Soy Broth is supplemented with Yeast Extract for optimum growth of *Listeria*.

Principles of the Procedure

Tryptone, Soytone and Yeast Extract provide nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Sodium Chloride maintains the osmotic balance of the medium. Phosphate acts as a buffer. Acriflavine HCl and Nalidixic Acid are added for selectivity and Cycloheximide is used to inhibit growth of saprophytic fungi.

Formula

Listeria Enrichment Broth

Formula Per Liter	
Bacto Tryptone	17 g
Bacto Soytone	3 g
Bacto Dextrose	2.5 g
Sodium Chloride	5 g
Potassium Phosphate, Dibasic	2.5 g
Bacto Yeast Extract	6 g
Cycloheximide	0.05 g
Acridine HCl	0.015 g
Nalidixic Acid	0.04 g
Final pH 7.3 ± 0.2 at 25°C	

Precautions

- For Laboratory Use.
- HARMFUL. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. (US) HARMFUL IF SWALLOWED. (EC)** Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Liver, Lungs, Nerves.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin,

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	3.61% solution, soluble in distilled or deionized water on boiling. Solution is light to medium yellowish amber with a faint green ring at the surface, clear to very slightly opalescent.
Prepared Medium:	Light yellowish amber, clear to very slightly opalescent.
Reaction of 3.61% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Listeria Enrichment Broth per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Enterococcus faecalis</i>	29212*	2,000-10,000	suppressed at 18- 24 hours
<i>Escherichia coli</i>	25922*	2,000-10,000	marked to complete inhibition
<i>Listeria monocytogenes</i>	19114	100-1000	good
<i>Saccharomyces pastorianus</i>	25923	2,000-10,000	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.

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.

- 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 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

Listeria Enrichment Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Test tubes with closures
Autoclave
Incubator (30°C)

Method of Preparation

- Suspend 36.1 grams in 1 liter of distilled or deionized water.
- Heat to boiling to dissolve completely.
- Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

- Collect food samples in sterile containers and transport immediately to the laboratory following recommended guidelines.
- Process each food sample, using procedures appropriate for that sample.

Test Procedure

For food samples, use recommended laboratory procedures for isolating *Listeria*.

Results

Refer to appropriate references and procedures for results.

References

- 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.
- Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low- and high-fat, frozen and refrigerated ground beef. *J. Food Prot.* **57**:969-974.
- Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma. Special Report. *J. Assoc. Off. Anal. Chem.* **70**:769-772.

- Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. *J. Food Prot.* **58**:604-608.
- Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. *J. Food Prot.* **55**:4-7.
- Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. *J. Food Prot.* **58**:244-250.
- Conner, D. E., R. E. Brackett, and L. R. Beuchat.** 1986. Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. *Appl. Environ. Microbiol.* **52**:59.
- Kramer, P. A., and D. Jones.** 1969. Media selective for *Listeria monocytogenes*. *J. Appl. Bacteriol.* **32**:381-394.
- Lovett, J., D. W. Frances, and J. M. Hunt.** 1987. *Listeria monocytogenes* in raw milk: detection, incidence and pathogenicity. *J. Food Prot.* **50**:188-192.
- McBride, M. E., and K. F. Girard.** 1960. A selective method for the isolation of *Listeria monocytogenes* from mixed bacterial populations. *J. Lab. Clin. Med.* **55**:153-157.

Packaging

Listeria Enrichment Broth	500 g	0222-17
	10 kg	0222-08

Bacto® Litmus Milk

Intended Use

Bacto Litmus Milk is used for differentiating microorganisms based on acid production and coagulation or proteolysis of casein.

Summary and Explanation

Litmus Milk has been used for many years to detect the metabolic activities of microorganisms in milk and to aid in the identification of bacterial species. It is especially useful in species differentiation within

the genus *Clostridium*.¹ This medium is also of value in the maintenance and propagation of lactic acid bacteria. The reactions of litmus milk are a valuable criterion in identification.¹

Principles of the Procedure

Skim milk is a source of nutrients. The addition of a litmus indicator to milk expands its usefulness as a differential medium. Litmus incorporated in milk is both a pH indicator and an oxidation-reduction indicator.

User Quality Control

Identity Specifications

Dehydrated Appearance: Grayish-purple, free-flowing, homogenous.

Solution: 10% solution, soluble in distilled or deionized. Solution is light purple and opaque.

Prepared tubes: Light purple and opaque

Reaction of 10% Solution at 25°C: pH 6.8 ± 0.2.

Cultural Response

Prepare Litmus Milk per label directions. Inoculate and incubate at 35 ± 2°C for up to 7 days.

ORGANISM	ATCC*	INOCULUM CFU	REACTION IN LITMUS MILK
<i>Bacillus subtilis</i>	6633	1,000-2,000	K, D
<i>Clostridium perfringens</i>	12924	1,000-2,000	R, C
<i>Lactobacillus casei</i>	7469	1,000-2,000	A, R, C

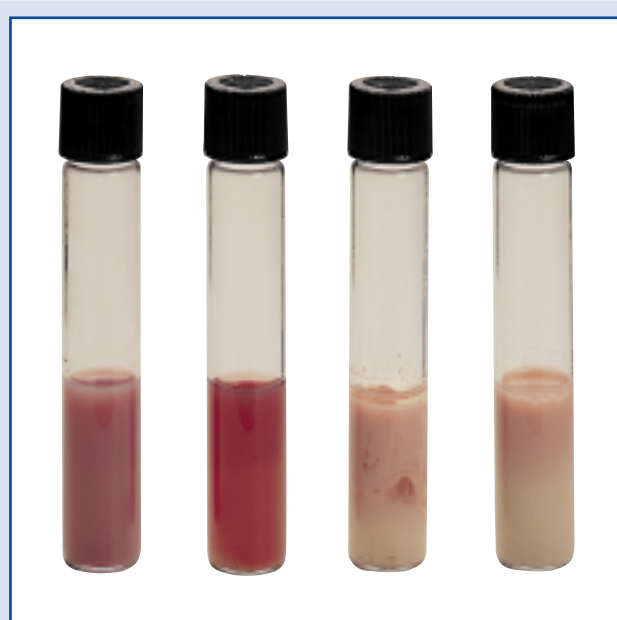
A = Acid reactions:
1. Pinkish-red medium.
2. Due to fermentation of the carbohydrates lactose and glucose.

K = Alkaline reactions:
1. Blue medium.
2. No fermentation of carbohydrates.
3. Organism attacks nitrogenous substances present in medium. The breakdown of lactalbumin by proteolytic enzymes form ammonia or basic amines.

R = Reduction:
1. White medium.
2. The enzyme reductase removes oxygen from the litmus, resulting in a decolorized, milky white appearance.
3. Reduction usually begins at the bottom of the tube.

C = Clot or Curd:
1. Milk protein coagulation.
2. Coagulation due to either a precipitation of casein by acid formation or the conversion of casein to paracasein by the enzyme rennin resulting in a clear watery fluid called "whey."

D = Digestion:
1. Milk protein digested.
2. Clearing of medium and dissolution of clot by digestion of casein.



Uninoculated tube *Bacillus subtilis* ATCC® 6633 *Clostridium perfringens* ATCC® 12924 *Lactobacillus casei* ATCC® 7469

Formula

Litmus Milk

Formula Per Liter	
Bacto Skim Milk	100 g
Bacto Litmus	0.75 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 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

Litmus Milk

Materials Required but not Provided

Glassware
Autoclave

Method of Preparation

1. Dissolve 100 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

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.

Limitations of the Procedure

1. Avoid overheating during sterilization, as the milk sugar will caramelize, resulting in discoloration giving an appearance atypical of the sterile medium.
2. During the sterilization period, Litmus Milk is reduced to a white colored base, however, upon cooling, the original color returns as oxygen is absorbed.
3. Reactions observed in Litmus Milk are not sufficient to speciate. Additional biochemical tests must be performed.¹

References

1. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol 1, p.275-284, Williams & Wilkins, Baltimore, MD.

Packaging

Litmus Milk	500 g	0107-17
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Bacto® Littman Oxgall Agar

Intended Use

Bacto Littman Oxgall Agar is used for isolating and cultivating fungi, especially dermatophytes.

Summary and Explanation

In 1947, Littman¹ described Littman Oxgall Agar which is neutral in reaction and suitable for growth of pathogenic fungi. It is a selective medium for the primary isolation of fungi.

Littman demonstrated that Littman Oxgall Agar is valuable for culturing the dermatophytes. Molds and yeasts form nonspreading, discrete colonies, that are easy to isolate in pure culture. He also suggested that the medium be used for estimating the normal fungal flora of feces, sputum and other human discharges. The medium can also be used for single cell isolation of fungi and plate counts of viable saprophytic fungi in foodstuffs and air.

In a comparative study, Littman² compared this medium with Sabouraud Dextrose Agar using a large variety of pathogenic and saprophytic fungi. He reported the isolation of three times as many fungi from feces, sputum, skin scrapings and hair on Littman Oxgall Agar and four times as many pathogenic dermatophytes on the selective medium compared with Sabouraud Dextrose Agar.

Principles of the Procedure

Bacto Peptone provides nitrogen, amino acids and carbon. Dextrose is an additional carbon source. Oxgall restricts the spreading of fungus colonies. Crystal Violet and streptomycin are selective bacteriostatic agents. Bacto Agar is a solidifying agent.

Formula

Littman Oxgall Agar

Formula Per Liter	
Bacto Peptone	10 g
Bacto Dextrose	10 g
Bacto Oxgall	15 g
Bacto Agar	20 g
Bacto Crystal Violet	0.01 g
Final pH 7.0 ± 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 breath 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.

- 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

Littman Oxgall Agar

Materials Required but not Provided

Glassware
Autoclave
Streptomycin

Method of Preparation

- Suspend 55 grams in 1 liter distilled or deionized water.
- Heat to boiling to dissolve completely.
- Autoclave at 121°C for 15 minutes.
- Cool to 46°C.
- Add 30 mcg streptomycin per ml of medium.

User Quality Control

Identity Specifications

Dehydrated Medium

Appearance: Grayish-blue, free-flowing, homogeneous.

Solution: 5.5% solution, soluble in distilled or deionized water on boiling. Solution is blue, very slightly to slightly opalescent.

Reaction of 5.5%
Solution at 25°C: pH 7.0 ± 0.2

Cultural Response

Prepare Littman Oxgall Agar per label directions with and without the addition of streptomycin. Inoculate and incubate plates at 25-30°C for up to 72 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH PLAIN	GROWTH w/STREPTOMYCIN
<i>Candida albicans</i>	10231	100-1,000	good	good
<i>Escherichia coli</i>	25922*	100-1,000	good	inhibited
<i>Saccharomyces cerevisiae</i>	9763	100-1,000	good	good
<i>Saccharomyces pastorianus</i>	9080	100-1,000	good	good
<i>Trichophyton mentagrophytes</i>	28185	100-1,000	fair to good	fair to 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

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

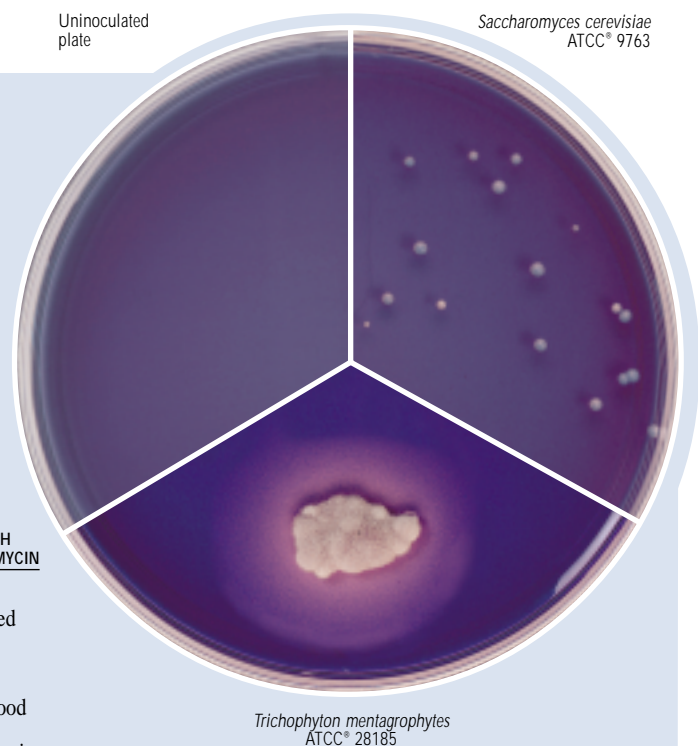
- Although culture techniques are primary in the identification of etiological agents of mycotic infections, they are not absolute. Often identification must be accomplished by using one or more of the following techniques to corroborate cultural findings: direct microscopic examination of the specimen; animal inoculation; biochemical determination; or serological procedures.
- Do not use to culture *Nocardia asteroides*, *Streptomyces*, or any other microorganism sensitive to streptomycin.³

References

- Littman, M. L.** 1947. Culture medium for primary isolation of fungi. *Science* **106**:109-111.
- Littman, M. L.** 1948. Growth of pathogenic fungi on a culture medium. *Am. J. Clin. Pathol.* **18**:409-420.
- MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 445-447. Williams & Wilkins, Baltimore, MD.

Packaging

Littman Oxgall Agar 500 g 0294-17



Bacto® Liver Infusion Agar

Bacto Liver Infusion Broth

Intended Use

Bacto Liver Infusion Agar is used for cultivating *Brucella* and other pathogenic organisms.

Bacto Liver Infusion Broth is used for cultivating a variety of organisms, particularly *Brucella* and anaerobes.

Summary and Explanation

Brucellosis is a zoonotic disease with a domestic animal reservoir. Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.¹

Most strains of *Brucella* will grow on chocolate or blood agar. However, special media such as liver infusion, tryptose, tryptone or brucella agar are preferred.² The nutritive factors of Liver Infusion media permit luxuriant growth of *Brucella* and other fastidious pathogens.

User Quality Control

Identity Specifications

Liver Infusion Agar

Dehydrated Appearance: Dark beige to light tan, free-flowing, homogeneous.

Solution: 5.5% solution, in distilled or deionized water on boiling, medium to dark amber, slightly opalescent to opalescent.

Prepared Medium: Medium to dark amber, slightly opalescent.

Reaction of 5.5% Solution at 25°C pH 6.9 ± 0.2

Liver Infusion Broth

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 3.5% solution, soluble in distilled or deionized water, medium to dark amber, clear to very slightly opalescent with a few particles.

Prepared Medium: Medium to dark amber, clear to very slightly opalescent with a few particles.

Reaction of 3.5% Solution at 25°C pH 6.9 ± 0.2

Cultural Response

Prepare Liver Infusion Agar and Liver Infusion Broth per label directions. Inoculate prepared medium and incubate under 5-10% CO₂ at 35 ± 2°C for 18-48 hours, or up to 72 hours if necessary. Incubate *Clostridium* under anaerobic conditions.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Brucella abortus</i>	4315	100-1,000	good
<i>Brucella melitensis</i>	4309	100-1,000	good
<i>Brucella suis</i>	4314	100-1,000	good
<i>Clostridium sporogenes</i>	11437	100-1,000	good

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

For isolating *Brucella* strains from contaminated milk, crystal violet (gentian violet) can be added to Liver Infusion Agar to suppress gram-positive organisms.³ Five percent (5%) heated horse or rabbit serum enhances growth of *Brucella*.⁴

Liver Infusion Agar at approximately half strength may be used to prepare Endamoeba medium for cultivating *Endamoeba histolytica*.⁵

Liver Infusion Broth maintains a degree of anaerobiosis well suited to support growth of anaerobic microorganisms, especially *Clostridium* species.

Principles of the Procedure

Infusion from Beef Liver and Proteose Peptone provide the nitrogen, amino acids, vitamins and carbon sources in Liver Infusion media. Sodium chloride maintains the osmotic balance. Bacto Agar is a solidifying agent.

Formula

Liver Infusion Agar

Formula Per Liter	
Beef Liver, Infusion from	500 g
Bacto Proteose Peptone	10 g
Sodium Chloride	5 g
Bacto Agar	20 g
Final pH 6.9 ± 0.2 at 25°C	

Liver Infusion Broth

Formula Per Liter	
Beef Liver, infusion from	500 g
Bacto Proteose Peptone	10 g
Sodium Chloride	5 g
Final pH 6.9 ± 0.2 at 25°C	

Precautions

- For Laboratory Use.
- Brucella* species are classified as Biosafety Level 3 pathogens. All manipulations with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).²
- Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

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

Expiration Date

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

Procedure

Materials Provided

Liver Infusion Agar
Liver Infusion Broth

Materials Required But Not Provided

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

Method of Preparation

- Liver Infusion Agar:** Suspend 55 grams in 1 liter distilled or deionized water and boil to dissolve completely.
Liver Infusion Broth: Dissolve 35 grams in 1 liter distilled or deionized water.
- Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
- Dispense Liver Infusion Agar into sterile Petri dishes or as desired. Dispense Liver Infusion Broth into sterile tubes or as desired.

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 *Brucella*, anaerobic microorganisms and other fastidious pathogens, refer to the procedures described in Bailey & Scott's Diagnostic Microbiology,⁴ Clinical Microbiology Procedures Handbook⁶ and Manual of Clinical Microbiology.⁷

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

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

References

- Moyer, N. P., and L. A. Holcomb.** 1995. *Brucella*, p. 549-555. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
- Carter, G. R.** 1979. Diagnostic procedures in veterinary bacteriology and mycology, 3rd ed. Charles C. Thomas, Springfield, IL.
- MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 802-806, vol. 1. Williams & Wilkins, Baltimore, MD.
- 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.
- Cleveland, L. R., and E. P. Sanders.** 1930. Encystation, multiple fission without encystment, encystation, metacystic development, and variation in a pure line and nine strains of *Entamoeba histolytica*. Arch. Protistenkd. **70**:223.
- Isenberg, H. D. (ed.).** 1995. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
- Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Liver Infusion Agar	500 g	0052-17
Liver Infusion Broth	500 g	0269-17
	10 kg	0269-08

Bacto® Liver Veal Agar

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	9.7% solution, soluble in distilled or deionized water upon boiling; medium to dark amber, opalescent, may have a slight precipitate.
Prepared Medium:	Medium to dark amber, opalescent; may have a slight precipitate.
Reaction of 9.7% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Liver Veal Agar per label directions. Inoculate medium and incubate at 35 ± 2°C under appropriate atmospheric conditions. Incubate clostridia anaerobically, *Neisseria* under increased CO₂, and streptococci aerobically.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Clostridium botulinum</i>	25763	100-1,000	good
<i>Clostridium tetani</i>	10779	100-1,000	good
<i>Neisseria meningitidis</i>	13090*	100-1,000	good
<i>Streptococcus pneumoniae</i>	6305	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.

Intended Use

Bacto Liver Veal Agar is used for cultivating anaerobic bacteria.

Summary and Explanation

Spray¹ described a procedure using the anaerobic culture dish for the cultivation of these organisms. Liver Veal Agar is identical to the medium described by Spray.² Liver Veal Agar provides a rich supply of nutrients for anaerobic and fastidious aerobic pathogens. The medium supports excellent growth of sporulating anaerobes and can be used for deep tube cultures.

Liver Veal Agar is specified in the FDA Bacteriological Analytical Manual (BAM)³ and Compendium of Methods for the Microbiological Examination of Food.⁴ Liver Veal Agar can be supplemented with 50% egg yolk for the cultivation of anaerobic organisms.⁵

Principles of the Procedure

Infusion from Liver, Infusion from Veal, Proteose Peptone, Neopeptone, Tryptone, Gelatin and Isoelectric Casein provide the rich nitrogen, amino acids and vitamin content of the medium. Soluble Starch is added to enhance the growth of anaerobes and Dextrose is a carbon source. Sodium chloride maintains osmotic balance and Bacto Agar is a solidifying agent.

Formula

Liver Veal Agar

Formula Per Liter	
Bacto Liver, Infusion from	50 g
Veal, Infusion from	500 g

Bacto Proteose Peptone	20 g
Bacto Gelatin	20 g
Bacto Soluble Starch	10 g
Isoelectric Casein	2 g
Bacto Dextrose	5 g
Bacto Neopeptone.....	1.3 g
Bacto Tryptone	1.3 g
Sodium Chloride	5 g
Sodium Nitrate	2 g
Bacto Agar	15 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

Liver Veal Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 97 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 anaerobic bacteria and other fastidious aerobic pathogens, refer to the procedure described in Clinical Microbiology Procedures Handbook⁶ and Manual of Clinical Microbiology.⁷

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. **Spray, R. S.** 1930. An improved anaerobic culture dish. *J. Lab. Clin. Med.* 16:203.
2. **Spray, R. S.** 1936. Semisolid media for cultivation and identification of the sporulating anaerobes. *J. Bacteriol.* 32:135.
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.
5. **Atlas, R. M.** 1993. Handbook of microbiological media. CRC Press, Boca Raton, FL.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. I. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Liver Veal Agar	500 g	0059-17
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Bacto® Loeffler Blood Serum

Intended Use

Bacto Loeffler Blood Serum is used for cultivating *Corynebacterium diphtheriae* from clinical specimens and in pure culture. The medium is also used for demonstrating pigment production and proteolysis.

Also Known As

Loeffler Blood Serum is also referred to as Loeffler's Serum Agar Medium, Loeffler's Coagulated Serum Slants and LAS.

Summary and Explanation

Loeffler Blood Serum is employed in the cultural diagnosis of diphtheria. Diphtheria is an acute infectious disease primarily of the

upper respiratory tract but occasionally of the skin.¹ It is caused by toxigenic strains of *Corynebacterium diphtheriae*, of which there are three biotypes: *mitis*, *intermedius*, and *gravis*.¹ The signs and symptoms of the disease are a pharyngeal membrane, sore throat, malaise, headache and nausea.² Death can result from respiratory obstruction by the membrane or myocarditis caused by the toxin.²

Loeffler Blood Serum is a modification of the horse serum, dextrose broth medium described by Loeffler³ for cultivating *C. diphtheriae*. This lipid-rich medium supports rapid growth of *C. diphtheriae* and is useful for demonstrating colonial pigmentation and the proteolytic activities of anaerobes and other microorganisms. Loeffler Blood Serum restores virulence and other identifying properties lost after prolonged incubation or repeated subculturing.⁴ Colonies grown on Loeffler medium exhibit excellent metachromatic granules under Gram stain.²

Cleveland and Sanders,⁵ and Spector⁶ used Loeffler Blood Serum in media for the cultivation of *Endamoeba histolytica*. Thompson⁷ hydrolyzed Loeffler Blood Serum with sodium hydroxide and added it to a citrate agar for the isolation of *C. diphtheriae*. On Thompson's⁷ medium, growth of diphtheria bacilli was stimulated while other respiratory flora were inhibited.

Principles of the Procedure

Beef Blood Serum provides the nitrogen, vitamins and amino acids necessary to support the growth of corynebacteria in Loeffler Blood Serum. Dextrose Broth is a source of fermentable carbohydrate and maintains the osmotic equilibrium of the medium.

Formula

Loeffler Blood Serum

Formula Per Liter

Beef Blood Serum	3 parts
Dextrose Broth	1 part
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 at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium beige, homogeneous, free-flowing.
8% Solution:	Soluble in distilled or deionized water warmed to 42-45°C.
Prepared Medium:	Coagulated in tubes - White to cream colored, slightly transparent at apex of slant.
Reaction of 8.0% Solution:	pH 7.1 ± 0.2 at 25°C

Cultural Response

Inoculate tubes with 30-300 CFU of test organism. Incubate 18-24 hours at 35 ± 2°C. Prepare slides from the growth, heat-fix, and stain with Methylene Blue, Loeffler. Stained cells will contain bipolar granules, club cells and some cells with general granulation.

ORGANISM	ATCC*	GROWTH
<i>Corynebacterium diphtheriae</i> type <i>mitis</i>	8024	good
<i>Corynebacterium diphtheriae</i> type <i>intermedius</i>	8032	good
<i>Corynebacterium diphtheriae</i> type <i>gravis</i>	8028	good

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

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

Loeffler Blood Serum

Materials Required But Not Provided

Glassware

Autoclave

Incubator

Method of Preparation

1. Suspend 80 grams in 1 liter distilled or deionized water warmed to 42-45°C. Check pH. Adjust to pH 7.1, if necessary.
2. Dispense into tubes having screw caps or other tightly sealing closures. Slant the tubes in the autoclave. Close the door loosely.
3. Coagulate the medium in constantly flowing steam for 10 minutes. Close the door tightly.
4. Autoclave at 121°C for 15 minutes.
5. Allow autoclave pressure to fall to zero before removing tubes.

Specimen Collection and Preparation

Both throat and nasopharyngeal specimens are necessary in cases of respiratory illness. If cutaneous diphtheria is suspected, collect skin, throat and nasopharynx specimens. Sterile silica gel is recommended for shipping clinical specimens when cultures are not obtained on site.¹

Test Procedure¹

1. If the swab appears desiccated, was collected several days prior to receipt, or is received in silica gel, place it into Todd-Hewitt Broth supplemented with 3% sterile rabbit blood. Incubate the culture overnight, then inoculate onto isolation media.
2. Inoculate the specimen onto cystine tellurite blood agar and blood agar plates, and streak for isolation on a Loeffler Blood Serum slant, leaving the swab on the slant during incubation.
3. Incubate aerobically at 35°C.
4. After 2-4 hours, prepare and heat fix a smear from the Loeffler Blood Serum slant. Flood the slide with Methylene Blue, Loeffler for 1 minute, rinse with tap water, and blot dry. Examine the smear for morphology typical of *C. diphtheriae*.
5. After 18-24 hours incubation, subculture onto a second plate of cystine tellurite blood agar.

Results

Examine all plates at 24-48 hours for colonies typical of *C. diphtheriae*. Subculture colonies that are catalase positive and exhibit typical morphology onto blood agar to provide growth for identification procedures.

Definitive identification of a *C. diphtheriae* isolate as a true pathogen requires demonstration of toxin production.⁸

For a complete discussion on the collection, isolation and identification of *Corynebacterium diphtheriae* and other *Corynebacterium* species, refer to the appropriate procedures.

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. Loeffler Blood Serum must be used in parallel with blood agar and a tellurite-containing medium (cystine tellurite agar or modified Tinsdale medium) for selection and differentiation of *Corynebacterium*.²
3. Metachromatic granules that take up methylene blue are characteristic of *Corynebacterium*; however, other microorganisms may also display stained granules (e.g., *Propionibacterium*, some *Actinomyces*, and pleomorphic streptococci strains) and resemble corynebacteria. Additional culture, biochemical identification, and toxigenicity tests must be performed for differentiation and identification.⁴

References

1. **Isenberg, H. D. (ed.)** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
2. **Clarridge, J. E., and C. A. Spiegel.** 1995. *Corynebacterium* and miscellaneous irregular gram-positive rods, *Erysipelothrix*, and

Gardnerella, p. 357-377. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

3. **Loeffler, F.** 1887. Darauf theilte HeuLoeffler en einem Zweiten Vortrag die ergebnisse seiner weiteren untersuchungen uber die Diphtherie-Bacillen mit. Zentralbl. Bacteriol. **2**:105.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 448-451, Williams & Wilkins, Baltimore, MD.
5. **Cleveland, L. R., and E. P. Sanders.** 1930. Encystation, multiple fission without encystment, metacystic development, and variation in a pure line and nine strains of *Entamoeba histolytica*. Arch. Protistenkd. **70**:223.
6. **Spector, B. K.** 1932. A comparative study of cultural and immunological methods of diagnosing infections with *Entamoeba histolytica*. J. Prevent. Med. **6**:117.
7. **Thompson.** 1929. J. Infect. Dis. **45**:163.
8. **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

Loeffler Blood Serum 500 g 0070-17*

*Store at 2-8°C

Bacto® Lowenstein Medium Base · Bacto Lowenstein Medium, Gruft · Bacto Lowenstein Medium, Jensen · Bacto Lowenstein Medium, Jensen Deeps · Bacto Lowenstein Medium w/5% NaCl

User Quality Control

Identity Specification

Lowenstein Medium Base

Dehydrated Appearance: Medium to dark green-blue, free flowing, homogenous.

Solution: 6.2% solution containing 2% glycerol, soluble in distilled or deionized water on boiling. Dark blue-green, opalescent, viscous.

Prepared Medium

(as Lowenstein Medium, Jensen): Pale green, smooth slant, opaque.

Lowenstein Medium, Gruft Tubes; Lowenstein Medium, Jensen Tubes; Lowenstein Medium w/5% NaCl

Appearance: Pale green, opaque, smooth slants.

Reaction of Medium at 25°C: pH 6.8-7.4

Lowenstein Medium, Jensen Deeps

Appearance: Pale green, opaque butts.

Reaction of Medium at 25°C: pH 7.2 ± 0.2

continued on following page

Intended Use

Bacto Lowenstein Media are prepared with fresh egg and glycerol to isolate, cultivate and differentiate mycobacteria.

Lowenstein Medium, Jensen Deeps are used for determining the catalase activity of mycobacteria.

Lowenstein Medium w/5% NaCl is used for differentiating mycobacteria on the basis of NaCl tolerance.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.¹ In 1985, the number of tuberculosis cases (TB) in the United States began increasing. Prior to this time, the number of US cases had been decreasing, reaching a low in 1984.² Non-tuberculous mycobacteria infections have also increased since 1985.³

The use of egg-based media for primary isolation of mycobacteria have the following significant advantages:

1. Egg-based media support a wide variety of mycobacteria.
2. The growth of mycobacteria on egg media can be used for niacin testing.

A disadvantage of egg-based media is that contaminating proteolytic organisms tend to liquefy the medium.³

Principles of the Procedure

Lowenstein formulations are egg-based media that contain a moderate amount of malachite green to suppress the growth of contaminating organisms. These media are commonly used in the clinical laboratory to isolate acid fast organisms from sterile and nonsterile sources.⁴

Bacto Lowenstein Medium, Jensen (LJ) is a modification of the Jensen formulation for Lowenstein Medium.⁵ It contains salts and a moderate concentration of malachite green to prevent the growth of most contaminants and to allow early growth of mycobacteria.

Lowenstein Medium, Gruft is the Gruft modification of Lowenstein Medium, Jensen.⁶ Ribonucleic acid is incorporated into the medium to increase the isolation of mycobacteria. Penicillin and Nalidixic Acid are added to decrease contamination.

The increased sodium chloride concentration in Lowenstein Medium, Jensen w/5% NaCl helps to differentiate rapid-growing mycobacteria from slow growers, which are inhibited in the presence of salt. Glycerol is added as a carbon source.

Formula

Lowenstein Medium Base

Formula Per Liter	
Bacto Asparagine	3.6 g
Monopotassium Phosphate	2.4 g
Magnesium Sulfate	0.24 g
Magnesium Citrate	0.6 g
Potato Flour	30 g
Malachite Green	0.4 g

Lowenstein Medium, Jensen

Formula Per Liter	
Bacto Asparagine	3.6 g
Monopotassium Phosphate	2.4 g
Magnesium Sulfate	0.24 g
Magnesium Citrate	0.6 g
Potato Flour	30 g
Malachite Green	0.4 g
Bacto Glycerol	12 ml
Distilled/Deionized Water	588 ml
Homogenized Egg	1,000 ml

Lowenstein Medium, Jensen Deeps (per 1600 ml)

Formula Per Liter	
Bacto Lowenstein Medium Base	37.2 g
Bacto Glycerol	12 ml
Distilled/Deionized Water	588 ml
Homogenized Egg	1,000 ml

Lowenstein Medium Gruft (per 1600 ml)

Formula Per Liter	
Bacto Lowenstein Medium Base	37.2 g
Bacto Glycerol	12 ml
Distilled/Deionized Water	588 ml
Homogenized Egg	1,000 ml
Penicillin	80,000 units
Nalidixic Acid	56 mg
Ribonucleic Acid	80 µg

Lowenstein Medium w/5% NaCl (per 1600 ml)

Formula Per Liter

Bacto Lowenstein Medium Base	37.2 g
Bacto Glycerol	12 ml
Distilled/Deionized Water	588 ml
Sodium Chloride	80 g
Homogenized Egg	1,000 ml

Precautions

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

Storage

Store Lowenstein Medium Base below 30°C.

Store prepared tubed 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 product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lowenstein Medium Base
 Lowenstein Medium, Gruft
 Lowenstein Medium, Jensen
 Lowenstein Medium, Jensen Deeps
 Lowenstein Medium w/5% NaCl

Materials Required But Not Provided

Flasks
 Distilled or deionized water
 Screw capped tubes
 Specimen digestant and decontaminant
 Centrifuge
 Inoculating Needles
 Incubator
 Fresh egg
 Autoclave
 Inspissator (optional)
 Water bath (optional)
 Glycerol
 Tween-Hydrogen Peroxide 1:1

Method of Preparation

Lowenstein Medium Base

1. Suspend 37.2 grams of Lowenstein Medium Base in 600 ml distilled or deionized water containing 12 ml of Glycerol and boil with constant agitation.
2. Autoclave at 121°C for 15 minutes. Cool to 45-60°C.
3. Aseptically add the sterile base to 1 liter of a uniform suspension of fresh eggs prepared under aseptic conditions. Swirl gently to avoid introducing air into the suspension.
4. Dispense into sterile screw cap tubes or bottles. Arrange in a slanted position.
5. Place in an inspissator, water bath or autoclave at 85°C for 45 minutes to coagulate the medium.

Prepared Lowenstein Media

Prepared media are ready to use.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Observe for colonies that may or may not be pigmented. Colony morphology depends on the species isolated.

Limitations of the Procedure

Negative culture results do not rule out an active mycobacterial infection. Some factors responsible for unsuccessful cultures are:

1. The specimen was not representative of the infectious material, i.e., saliva instead of sputum.
2. The mycobacteria were destroyed during digestion and decontamination of the specimen.
3. Gross contamination interfered with the growth of mycobacteria.
4. Proper aerobic and increased CO₂ tension were not provided during incubation.

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria:

molecular genetic insights. *Clinical Microbiology Reviews* 8:496-514.

2. **Kleitmann, W.** 1995. Resistance and susceptibility testing for *Mycobacterium tuberculosis*. *Clinical Microbiology 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. Tenover (ed.), *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.
5. **Enter. Bacteriol. Parasitnek.** 1932. Abt. 1, 125:222.
6. **J. Bact.** 1965. 90:829.

Packaging

Lowenstein Medium Base	500 g	0444-17
	2 kg	0444-07
Lowenstein Medium, Gruft	20 tubes	1417-39
	100 tubes	1417-79
Lowenstein Medium, Jensen	20 tubes	1017-39
	100 tubes	1017-79
	100 x 1 oz	1017-57
Lowenstein Medium w/5% NaCl	20 tubes	1423-39
Lowenstein Medium, Jensen Deeps	20 tubes	1289-76

Cultural Response

Lowenstein Medium Base; Lowenstein Medium, Gruft Tubes; Lowenstein Medium, Jensen Tubes

Prepare Lowenstein Medium Base (as Lowenstein Medium, Jensen) per label directions or use prepared tubes. Inoculate and incubate at 35°C under CO₂ for up to three weeks.

ORGANISM	ATCC*	INOCULUM CFU	RECOVERY
<i>Escherichia coli</i> †	25922*	1,000-2,000	partial inhibition
<i>Mycobacterium fortuitum</i>	6841	100-300	good
<i>Mycobacterium intracellulare</i>	13950	100-300	good
<i>Mycobacterium kansasii</i>	12478	100-300	good
<i>Mycobacterium scrofulaceum</i>	19981	100-300	good
<i>Mycobacterium tuberculosis</i> H37Ra	25177	100-300	good

†Tested on Lowenstein Medium, Gruft, only

Lowenstein Medium w/5% NaCl

Inoculate and incubate at 35°C under CO₂ for up to three weeks.

ORGANISM	ATCC*	INOCULUM CFU	RECOVERY
<i>Mycobacterium smegmatis</i>	14468	100-300	good
<i>Mycobacterium tuberculosis</i>	25177	100-300	inhibited

Lowenstein Medium, Jensen Deeps

Inoculate and incubate at 35°C under CO₂ for 2 weeks. Cap loosely for 7 days, then tighten cap and incubate 1 week longer. Add 1 ml of Tween-Hydrogen Peroxide reagent to the 2-week culture. Measure the height of the column of bubbles after 5 minutes. (Tween-Hydrogen Peroxide reagent is a 1:1 final solution of 30% hydrogen peroxide in distilled water and sterile, cooled 10% Tween® 80 in distilled water.)

ORGANISM	ATCC*	INOCULUM CFU	RECOVERY
<i>Mycobacterium gordonae</i>	14470	100-300	greater than 45 mm
<i>Mycobacterium tuberculosis</i>	25177	100-300	less than 45 mm



Uninoculated tube

Mycobacterium fortuitum
ATCC® 6841

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® Luria Agar Base, Miller

Intended Use

Bacto Luria Agar Base, Miller is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures with or without added glucose.

Summary and Explanation

Luria Agar Base, Miller, a nutritionally rich medium designed for growth of pure cultures of recombinant strains, is based on the Luria Broth Agar formula described by Miller.¹ *E. coli* is grown to late log phase in LB Medium. Some plasmid vectors replicate to a high copy number and do not require selective amplification. Some vectors do not replicate so freely and need to be selectively amplified. Chloramphenicol may be added to inhibit host synthesis and, as a result, prevent replication of the bacterial chromosome.²

Luria Agar Base, Miller contains one tenth and one twentieth, respectively, the sodium chloride level of the LB Agar, Lennox and LB Agar, Miller formulations.¹⁻³ This allows the researcher to select the optimal salt concentration for a specific strain. The medium may be aseptically supplemented with glucose, if desired.

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride. Bacto Agar is the solidifying agent.

Formula

Luria Agar Base, Miller

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	0.5 g
Bacto Agar	15 g
Final pH	7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free flowing, homogeneous.
Solution:	3.05% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Medium:	Very light amber, slightly opalescent.
Reaction of 3.05% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Luria Agar Base, Miller with 10 ml sterile 20% glucose solution per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Escherichia coli</i>	33526	100-300	Good

The culture listed is the minimum that should be used for performance testing.

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 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

Luria Agar Base, Miller

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)
20% glucose solution (optional)

Method of Preparation

1. Suspend 30.5 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 in a waterbath.
4. If desired, aseptically add 10 ml sterile 20% glucose solution and mix thoroughly.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.^{1,2}

Results

Growth is evident in the form of isolated colonies and/or a confluent lawn on the surface of the medium.

References

1. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
3. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology*. **1**:190-206.

Packaging

Luria Agar Base, Miller	500 g	0413-17
	2 kg	0413-0

Bacto® Lysine Iron Agar

Intended Use

Bacto Lysine Iron Agar is used for differentiating microorganisms, especially *Salmonella*, based on lysine decarboxylation/deamination and H₂S production.

Summary and Explanation

Lysine Iron Agar is prepared according to the formulation of Edwards and Fife¹, who developed the medium to detect *Salmonella arizonae* (formerly *Arizona arizonae*). Because *S. arizonae* ferments lactose so rapidly, the authors found that the expected H₂S production on triple sugar iron agar was suppressed. Since *S. arizonae* strains are found occasionally in outbreaks of food borne infection, it is important to be able to detect them. By eliminating lactose and incorporating lysine, Edwards and Fife devised a medium that differentiates enteric bacilli based on their ability to decarboxylate or deaminate lysine and produce abundant hydrogen sulfide. The medium is especially recommended for detecting rapid lactose-fermenting *S. arizonae*. It is specified in Standard Methods for *Salmonella* testing.^{2,3,4,5,6}

Principles of the Procedure

Lysine Iron Agar contains Bacto Peptone which provides carbon and nitrogen sources required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth, as well as additional sources of nitrogen and carbon. Dextrose is an energy source. L-Lysine Hydrochloride is the substrate used to detect the lysine decarboxylase and lysine deaminase enzymes. Ferric Ammonium Citrate and Sodium Thiosulfate are indicators of hydrogen sulfide

production. Brom Cresol Purple, a pH indicator, is yellow at or below pH 5.2 and purple at or above pH 6.8. Bacto Agar is a solidifying agent.

Formula

Lysine Iron Agar

Formula Per Liter

Bacto Peptone	5	g
Bacto Yeast Extract	3	g
Bacto Dextrose	1	g
L-Lysine Hydrochloride	10	g
Ferric Ammonium Citrate	0.5	g
Sodium Thiosulfate	0.04	g
Bacto Brom Cresol Purple	0.02	g
Bacto Agar	15	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.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.45% solution, soluble in distilled or deionized water upon boiling. Solution is reddish-purple, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Purple, slightly opalescent without precipitate.
Reaction of 3.45% Solution at 25°C:	pH 6.7 ± 0.2

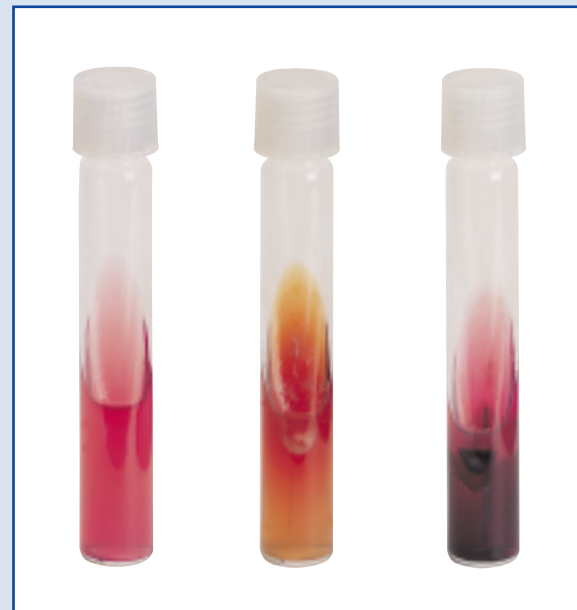
Cultural Response

Prepare Lysine Iron Agar per label directions. Inoculate with undiluted cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC*	GROWTH	LYSINE DECARBOXYLATION (BUTT)	LYSINE DEAMINATION (SLANT)	H ₂ S (APEX OF SLANT)
<i>Proteus mirabilis</i>	25933	good	- yellow	+ red	-
<i>Salmonella arizonae</i>	13314	good	+ purple	- purple	+ black
<i>Salmonella typhimurium</i>	14028*	good	+ purple	- purple	+ black

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

*Available as Bactrol™ Disks; use as directed in Bactrol Disks Technical Information.



Uninoculated tube

Proteus mirabilis
ATCC® 25933

Salmonella typhimurium
ATCC® 14028

Procedure

Materials Provided

Lysine Iron Agar

Materials Required But Not Provided

Glassware

Autoclave

Incubator (35°C)

Inoculating needle

Method of Preparation

1. Suspend 34.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes. Autoclave at 121°C for 12 minutes.
4. Allow medium to cool in a position that will provide a short slant and a deep butt.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Using a straight needle, pick the center of a well-isolated colony from a fresh, pure culture.
2. Inoculate by stabbing to the base of the butt and streaking the slant.
3. Cap the tube loosely to ensure aerobic conditions.
4. Incubate at 35°C for 18-48 hours.
5. Examine at 18-24 and 40-48 hours for growth and color changes in the butt and the slant of the medium and for blackening at the apex of the slant.

Results

Lysine decarboxylase reaction:

Positive: Purple (alkaline) butt, purple slant.

Negative: Yellow (acid) butt, purple (alkaline) slant.

Lysine deaminase reaction:*

Positive: Red slant.

Negative: Purple slant.

Hydrogen sulfide reaction:

Positive: Blackened medium at the apex of the slant.

* *Proteus* and *Providencia* cultures produce a red slant over a yellow (acid) butt.

Limitations of the Procedure

1. *Salmonella paratyphi A*, unlike other *Salmonella*, does not produce lysine decarboxylase and so produces an alkaline slant and an acid butt.
2. H₂S-producing *Proteus* species do not blacken the medium.^{2,7} It is, therefore, suggested that Lysine Iron Agar be used in conjunction with Triple Sugar Agar or other media to confirm differentiation.
3. The reaction of *Morganella morganii* may be variable after 24 hours incubation and may require longer incubation.⁷

References

1. **Edwards, P. R., and M. A. Fife.** 1961. Lysine-iron agar in the detection of *Arizona* cultures. *Appl. Microbiol.* **9**:478.
2. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
3. **Russell, S. F., J. -Y. D'Aoust, W. H. Andrews and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. In C. Vanderzant, and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1992. Pathogens in milk and milk products, p. 103-212. In R. T. Marshall, (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.
6. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. In Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Finegold, S. M., and W. J. Martin.** 1982. Bailey and Scott's diagnostic microbiology, 6th ed., p. 631. The CV Mosby Company, St. Louis, MO.

Packaging

Lysine Iron Agar	100 g	0849-15
	500 g	0849-17

Bacto® Lysine Medium

Intended Use

Bacto Lysine Medium is used for isolating and enumerating "wild" yeast contaminants in brewery pitching yeasts.

Summary and Explanation

Walters and Thiselton¹ formulated a liquid synthetic medium containing lysine to study brewery yeasts. The medium separated yeasts into two groups based on their ability to grow using L-lysine as a sole source of nitrogen. Strains of brewery culture yeasts, *Saccharomyces cerevisiae* and *S. carlsbergensis*, grew poorly or not at all in the medium (lysine

negative) while yeasts known as "wild" contaminants, including *S. pastorianus* and *S. cerevisiae* var. *turbidans*, grew (lysine positive).

Morris and Eddy² modified the formula by adding agar and confirmed the work of Walters and Thiselton using quantitative results. They showed a low level of infection of a pitching yeast with wild yeasts could be determined. They also recommended the addition of antibiotics to the medium for samples heavily contaminated with bacteria.

Principles of the Procedure

Lysine Medium contains Dextrose as the carbohydrate. L-Lysine is the nitrogen source. Essential vitamins, salts, amino acids and other nutrients are present in defined amounts. Bacto Agar is the solidifying agent.

Formula

Lysine Medium

Formula Per Liter	
Bacto Dextrose	44.5 g
Potassium Dihydrogen Phosphate	1.78 g
Magnesium Sulfate	0.89 g
Calcium Chloride	0.178 g
Sodium Chloride	0.089 g
Adenine	0.00178 g
DL-Methionine	0.000891 g
L-Histidine	0.000891 g
DL-Tryptophan	0.000891 g
Boric Acid	0.000089 g
Zinc Sulphate	0.0000356 g
Ammonium Molybdate	0.0000178 g
Manganese Sulphate	0.0000356 g
Ferrous Sulphate	0.0002225 g
Inositol	0.02 g
Calcium Pantothenate	0.002 g
Aneurine	0.0004 g
Pyridoxine	0.0004 g
p-Aminobenzoic Acid	0.0002 g
Nicotinic Acid	0.0004 g
Riboflavin	0.0002 g
Biotin	0.000002 g
Folic Acid	0.000001 g
L-Lysine	1 g
Bacto Agar	17.5 g
Final pH 4.8 ± 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: 6.6% solution containing 1 ml 50% potassium lactate solution per 100 ml medium, soluble in distilled or deionized water on gentle boiling; very light amber, very slightly to slightly opalescent without significant precipitate.

Prepared Medium: Very light amber, slightly opalescent without significant precipitate.

Final reaction of pH-adjusted
6.6% solution at 25°C: pH 4.8 ± 0.2

Cultural Response

Prepare Lysine Medium per label directions. Inoculate and incubate at 25 ± 2°C for 72 hours.

ORGANISM	ATCC*	INOCULUM CFU	GROWTH
<i>Pichia fermentans</i>	10651	100-1,000	good
<i>Saccharomyces pastorianus</i>	2700	100-1,000	none to fair

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

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

Lysine Medium

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (25°C)
10% Lactic acid solution
50% Potassium lactate solution

Method of Preparation

1. Suspend 6.6 grams in 100 ml distilled or deionized water containing 1 ml 50% potassium lactate solution.
2. Boil gently to dissolve completely.
3. Cool to 50°C.
4. Adjust to final pH using 10% lactic acid, if necessary.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Wash and centrifuge the sample of pitching yeast three times with distilled water.
2. Resuspend the pellet in distilled water to contain approximately 10⁷ cells per ml.
3. Spread 0.2 ml of the cell suspension over the surface of the prepared medium.
4. Incubate plates at 25°C. Examine daily for growth.

Results

Count the number of colonies that develop and express the degree of contamination as the number of wild cells per million cells of inoculum.

Limitations of the Procedure

1. Use of a test inoculum having less than 10⁴ cells may permit growth of brewing yeast cells that can be confused with growth of wild yeasts. Use of an inoculum that exceeds 10⁴ cells will restrict growth of the unwanted brewing yeasts to tiny microcolonies.²

References

1. Walters, L. S., and M. R. Thiselton. 1953. J. Inst. Brew. 59:401-404.
2. Morris, E. O., and A. A. Eddy. 1957. J. Inst. Brew. 63:34-35.

Packaging

Lysine Medium

500 g

1894-17