Bacto® M9CA Medium

Intended Use

Bacto M9CA Medium is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

M9CA is based on M9 Minimal Salts[1] but with the addition of casamino acids. The medium may be supplemented with an appropriate carbon and energy source, such as dextrose. The Casamino Acids provide nitrogen in a readily available form. The medium will support the growth of "wild-type" and recombinant strains of *E. coli*. M9CA contains salts that supply nitrogen, phosphorus, and trace minerals.

Principles of the Procedure

Casamino Acids make this a richer medium than M9 Minimal Salts, providing all of the amino acids except tryptophan. Ammonium Chloride provides a source of nitrogen. Sodium and Potassium Phosphates buffer against pH changes due to carbohydrate metabolism. Dextrose, aseptically added to the medium, is a carbon and energy source. Magnesium Sulfate is a source of magnesium ions required in a variety of enzymatic reactions, including DNA replication.

Formula

M9CA Medium

Formula Per Liter

Bacto Casamino Acids	4 g
Sodium Phosphate, Dibasic, Anhydrous	6.8 g
Potassium Phosphate, Monobasic	3 g
Sodium Chloride	0.5 g
Ammonium Chloride	1 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

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

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.53% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear, no significant precipitate.
Reaction of 1.53% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare M9CA Medium per label directions. Inoculate and incubate at 35°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (B)	23226	100-300	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 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

M9CA Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Sterile 20% glucose solution
Sterile 1.0 M $MgSO_4$ solution
Incubator (35°C)

Method of Preparation

1. Dissolve 15.3 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. After cooling to below 50°C, aseptically add 20 ml of filter-sterilized 20% glucose solution and 2 ml of filter-sterilized 1M Magnesium Sulfate solution. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.

Results

Growth is evident in the form of turbidity.

References

1. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Packaging

M9CA Medium	500 g	0454-17
Dextrose	500 g	0155-17

Bacto® M9 Minimal Salts, 5x

Intended Use

Bacto M9 Minimal Salts, 5x is used in preparing M9 Minimal Medium which is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

M9 Minimal Salts, 5x is a 5x concentrate that is diluted to a 1x concentration and supplemented with an appropriate carbon and energy source, such as dextrose, to provide a minimal, chemically defined medium. The medium will support the growth of "wild-type" strains of *E. coli*. M9 Minimal Salts is useful for maintaining positive selection pressure on plasmids coding for the ability to produce essential substances such as amino acids or vitamins. M9 Minimal Medium is also used to maintain stocks of F´ containing bacteria for use with M13. The medium can be supplemented with specific amino acids or other metabolites, allowing for selection of specific auxotrophs.

Principles of the Procedure

Sodium Phosphate and Potassium Phosphate are present as buffering agents. Ammonium Chloride is a source of nitrogen for cellular systems. Sodium Chloride maintains isotonicity in the final medium. Glucose may be added as a source of carbohydrate. Supplementing the medium with magnesium and calcium increases the growth of recombinants.

Formula

M9 Minimal Salts, 5x

Formula Per Liter

Sodium Phosphate, Dibasic, Anhydrous	33.9 g
Potassium Phosphate, Monobasic	15 g
Sodium Chloride	2.5 g
Ammonium Chloride	5 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	5.64% solution, soluble in distilled or deionized water. Solution is colorless, clear.
Reaction of 5.64% Solution (5x concentrate) at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare M9 Minimal Salts, 5x and dilute to 1x. Supplement with glucose per label directions. Inoculate and incubate at 35°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	23226	30-300	Good
Escherichia coli	39403	30-300	Good

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

Precautions

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

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

M9 Minimal Salts, 5x

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Sterile 20% glucose solution
Sterile 1.0 M $MgSO_4$ solution
Sterile 1.0 M $CaCl_2$ solution (optional)
Incubator (35°C)

Method of Preparation

1. Dissolve 56.4 grams in 1 liter of distilled or deionized water. It is recommended that this liter be separated into 200 ml aliquots.
2. Autoclave at 121°C for 15 minutes.
3. To prepare M9 Minimal Medium, add 200 ml sterile M9 Minimal Salts, 5x to 750 ml sterile distilled or deionized water, which has been cooled to 45-50°C. Adjust final volume to 1 liter.
4. Aseptically add 20 ml filter-sterilized 20% glucose solution, 2 ml sterile 1.0 M magnesium sulfate ($MgSO_4$) solution and, if desired, 0.1 ml sterile 1.0 M calcium chloride ($CaCl_2$) solution. Mix well.
5. If desired, supplement with amino acids, as appropriate.

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 should be evident by the appearance of turbidity.

References

1. **Davis, L. G., M. D. Dibner, and J. F. Battey.** 1986. Basic methods in molecular biology. Elsevier, New York, N.Y.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Packaging

M9 Minimal Salts, 5x	500 g	0485-17

Bacto® M17 Broth
Bacto M17 Agar

User Quality Control

Identity Specifications

M17 Broth

Dehydrated Appearance:	Beige to medium tan, free-flowing, homogeneous.
Solution:	3.725% solution, soluble in distilled or deionized water. Solution is light-medium to medium amber, clear to very slightly opalescent.
Prepared Medium:	Light medium to medium amber, clear to very slightly opalescent, no significant precipitate.
Reaction 3.725% Solution at 25°C:	pH 6.9 ± 0.2

M17 Agar

Dehydrated Appearance:	Beige to medium tan, free-flowing, homogeneous.
Solution:	4.825% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent, no significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent, no significant precipitate.
Reaction of 4.825% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare Nutrient Gelatin per label directions. Using a heavy inoculum, inoculate by stabbing the tube and incubate at 35 ± 2°C for 18-48 hours or up to two weeks, if required. To read gelatinase, refrigerate until well chilled and compare to uninoculated tube. Tilt tubes carefully to test for liquefaction. Tubes positive for gelatinase remain liquid.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus delbrueckii subsp. *bulgaricus*	11842	100-1,000	none to poor
Lactococcus lactis subsp. *cremoris*	9625	100-1,000	good
Streptococcus thermophilus	19258	100-1,000	good

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

Intended Use

Bacto M17 Broth is used for isolating and enumerating lactic streptococci from yogurt, cheese starters and other dairy products.

Bacto M17 Agar is used for enumerating lactic streptococci in yogurt, cheese starters and other dairy products.

Summary and Explanation

Lactic streptococci are acid-producing bacteria. They are nutritionally fastidious and require complex culture media for optimum growth. One study showed that in a synthetic medium, all strains had an obligate requirement for at least six amino acids and three vitamins.[1] These homofermentative lactic streptococci produce large amounts of acid and, in a culture medium without an adequate buffering system, the pH decreases and adversely affects growth. Lowrie and Pearce[2] developed M16 Medium but it lacked a strong buffering system. Terzaghi and Sandine[3] worked with M16 Medium and demonstrated that the rapid drop in pH that accompanies lactic streptococcal growth can adversely affect colony size and phage plaque formation. They modified M16 Medium using disodium-ß-glycerophosphate as a buffer and called it M17.

Shankar and Davies[4] found that disodium-ß-glycerophosphate in M17 Broth suppressed *Lactobacillus bulgaricus* and selectively isolated *Streptococcus thermophilus* from yogurt. Similar results were achieved using M17 Broth solidified with agar. The International Dairy Federation recommends M17 Agar for isolating *S. thermophilus* from yogurt.[5] M17 Agar is a standard methods medium for isolating lactic streptococci.[6]

Principles of the Procedure

M17 Broth and M17 Agar contain Tryptone, Soytone, and Meat Digest as sources of carbon, nitrogen, vitamins and minerals. Yeast Digest supplies B-complex vitamins which stimulate bacterial growth. Disodium-ß-Glycerophosphate buffers the medium as acid is produced from fermentation of lactose. Ascorbic Acid stimulates growth of lactic streptococci. Magnesium Sulfate provides essential ions for growth. Bacto Agar is the solidifying agent in M17 Agar.

Formula

M17 Broth

Formula Per Liter

Bacto Tryptone	5 g
Bacto Soytone	5 g
Meat Digest	5 g
Yeast Digest	2.5 g
Ascorbic Acid	0.5 g
Magnesium Sulfate	0.25 g
Disodium-ß-glycerophosphate	19 g

Final pH 6.9 ± 0.2 at 25°C

M17 Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Soytone	5 g
Meat Digest	5 g
Bacto Yeast Extract	2.5 g
Ascorbic Acid	0.5 g
Magnesium Sulfate	0.25 g
Disodium-ß-glycerophosphate	19 g
Bacto Agar	11 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

M17 Broth or
M17 Agar

Materials Required but not Provided

Glassware
Petri Dishes (for M17 Agar)
Distilled or deionized water
Autoclave
Incubators (30°C and 35°C)

Method of Preparation

1. M17 Broth: Dissolve 37.25 grams in 950 ml distilled or deionized water.
   M17 Agar: Suspend 48.25 grams in 950 ml distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes.
3. Cool to 50°C.
4. Add 50 ml sterile 10% lactose solution. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Reiter, B., and J. D. Oram**. 1962. Nutritional studies on cheese starters. I. vitamin and amino acid requirements of single strain starters. J. Dairy Res. **29**:63-77.
2. **Lowrie and Pearce**. 1971. J. Dairy Sci. Technol. **6**:166.
3. **Terzaghi, B. E., and W. E. Sandine**. 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29**:807-813.
4. **Shankar, P. A., and F. L. Davies**. 1977. A note on the suppression of *Lactobacillus bulgaricus* in media containing ß-glycerophosphate and application of such media to selective isolation of *Streptococcus thermophilus* from yogurt. J. Soc. Dairy Tech. **30**:28-30.
5. **International Dairy Federation**. 1981. Identification and enumeration of micro- organisms in fermented milks. Joint IDF/ISO/AOAC Group E44.
6. **Vedamuthu, E. R., M. Raccach, B. A. Glatz, E. W. Seitz, and M. S. Reddy.** 1992. Acid-producing microorganisms, p. 225-238. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

M17 Broth	500 g	1856-17
M17 Agar	500 g	1857-17

Bacto® M Broth

Intended Use

Bacto M Broth is used for cultivating *Salmonella* in foods and feeds by the accelerated enrichment serology (ES) procedure.

Summary and Explanation

M Broth, prepared according to the formula of Sperber and Diebel[1], contains all the nutrients necessary for good growth and flagella development of *Salmonella*.

Fantasia, Sperber and Deibel[2] compared the enrichment serology (ES)' procedure with the traditional procedure outlined in the Bacteriological Analytical Manual[3] (BAM) and reported excellent agreement between the two. They found the ES procedure not only to be faster and less complicated but also as accurate and sensitive as the BAM procedure.

M Broth also conforms to the testing standards recommended by Compendium of Methods for the Microbiological Examination of Foods[4] (APHA) for the isolation and identification of foodborne *Salmonella*.

Both monoclonal and polyclonal enzyme immunoassay (EIA) methods have been described in AOAC Official Methods of Analysis[5] using M Broth. These methods are screening procedures for the presence of *Salmonella* and positive results must be confirmed by culture.

Principles of the Procedure

Yeast Extract is a source of B-complex vitamins. Tryptone provides organic nitrogen. D- Mannose and Sodium Citrate are fermentation energy sources. Mannose prevents fimbrial agglutination.[1] Sodium Chloride helps maintain osmotic equilibrium, while Dipotassium Phosphate acts as a buffer. The inorganic salts stimulate bacterial growth. Tween® 80 is a surfactant and dispersing agent.

Formula

M Broth

Formula per liter

Bacto Yeast Extract	5 g
Bacto Tryptone	12.5 g
Bacto D-Mannose	2 g
Sodium Citrate	5 g
Sodium Chloride	5 g
Dipotassium Phosphate	5 g
Manganese Chloride	0.14 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Tween® 80	0.75 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 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

M Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Lactose Broth
Selenite Cystine Broth
Tetrathionate Broth
Salmonella H Antisera Spicer-Edwards Set
Salmonella H Antiserum Poly D
Salmonella H Antiserum z_6
Normal saline
NaCl
Formalin
50°C waterbath

Method of Preparation

1. Suspend 36.2 grams in 1 liter distilled or deionized water.
2. Heat to boiling for 1-2 minutes, stirring carefully.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Prepare a 10% suspension of the test sample in Lactose Broth. Incubate at 35 ± 2°C for 18-24 hours.
2. Transfer 1 ml of the above preenrichment culture to 9 ml of Selenite Cystine Broth and 1 ml to 9 ml of Tetrathionate Broth. Incubate both enrichment media at 35 ± 2°C for 24 hours.

User Quality Control

Identity Specifications

Dehydrated appearance:	Beige, homogeneous with a tendency to lump.
Solution:	3.62% solution, soluble in distilled or deionized water on boiling for 1-2 minutes. Solution is light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 3.62% Solution at 25°C:	pH 7.0 ± 0.2

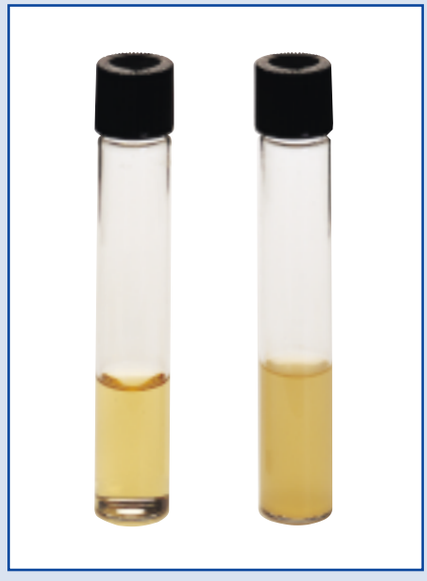
Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Salmonella choleraesuis	12011	100-1,000	good
Salmonella typhimurium	14028*	100-1,000	good

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

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



Uninoculated tube — *Salmonella choleraesuis* ATCC® 12011

3. Inoculate one 10 ml tube of M Broth, tempered to 35°C, with one drop from each of the above cultures. Incubate at 35 ± 2°C for 6-8 hours.
4. Prepare a formalin-salt solution by adding 4.2 grams of NaCl and 3 ml of formalin to 100 ml of distilled water. Place one drop in each of two Kahn tubes.
5. Carefully insert a pipette about 1 inch below the surface of the M Broth culture and transfer 0.85 ml of culture to each of the above Kahn tubes containing formalin-salt solution.
6. Prepare a pooled antiserum by combining together 0.5 ml each of rehydrated Salmonella H Antiserum Poly D and Salmonella H Antiserum z_6 (Salmonella H Antisera Spicer-Edwards Set) in 11.5 ml of 0.85% NaCl.
7. Add 0.1 ml pooled Salmonella H Antiserum to one of the Kahn tubes (above). Add 0.1 ml 0.85% NaCl solution to the other tube. Shake the tubes gently. Incubate in a 50°C water bath for 1 1/2 hours.

Results

Agglutination in the Kahn tube containing antiserum indicates the presence of *Salmonella*. Agglutination in the Kahn tube containing 0.85% NaCl solution (control tube) indicates a rough culture which should be streaked for isolation, passed through Motility GI Medium to enhance flagella, and then retested with pooled antiserum.

Alternative Testing Procedures

Refer to AOAC International[5] for screening procedures using enzyme immunoassay or DNA hybridization to detect *Salmonella* antigen in test samples.

References

1. **Sperber, W. H. and R. H. Deibel.** 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. Appl. Microbiol. **17:**533-539.
2. **Fantasia, L. D., W. H. Sperber, and R. H. Deibel.** 1969. Comparison of two procedures for detection of *Salmonella* in food, feed, and pharmaceutical products. Appl. Microbiol. **17:**540-541.
3. **Bacteriological Analytical Manual, 2nd ed.** 1969. US HEW, Washington, D.C.
4. **Flowers, R. S., J.- Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. *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. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

M Broth	500 g	0940-17
	2 kg	0940-07

Bacto® MIL Medium

Intended Use

Bacto MIL Medium is used for differentiating *Enterobacteriaceae* based on motility, lysine decarboxylation, lysine deamination and indole production.

Also Known As

MIL Medium conforms with Motility-Indole-Lysine Medium.

Summary and Explanation

MIL Medium, prepared according to the formula of Reller and Mirrett,[1] is a single culture medium that provides four differentiating biochemical reactions. When used in conjunction with Triple Sugar Iron Agar (TSI) and Urea Agar, as many as nine reactions are provided. This combination enables reliable initial identification of *Enterobacteriaceae*.[2,3] Extensive testing of 890 enteric cultures by Reller and Mirrett[1] gave essentially the same results with MIL Medium as with the standard motility, indole and lysine decarboxylase (Moeller) test media.

Principles of the Procedure

Bacto Peptone and Tryptone provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth. Lysine Hydrochloride is present as a substrate to detect lysine decarboxylase or lysine deaminase activity. Dextrose is an energy source. Ferric Ammonium Citrate is an H_2S indicator. Brom Cresol Purple is a pH indicator. Bacto Agar is a solidifying agent.

Formula

MIL Medium

Formula Per Liter

Bacto Peptone	10 g
Bacto Tryptone	10 g
Bacto Yeast Extract	3 g
L-Lysine Hydrochloride	10 g
Bacto Dextrose	1 g
Ferric Ammonium Citrate	0.5 g
Bacto Brom Cresol Purple	0.02 g
Bacto Agar	2 g

Final pH 6.6 ± 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

MIL Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
13 x 100 mm Screw-capped test tubes
Inoculating needle
SpotTest™ Indole Reagent Kovacs

Method of Preparation

1. Suspend 36.5 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense 5 ml amounts into 13 x 100 mm screw-capped test tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Using an inoculating needle, stab tubes with growth from an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C for 18-24 hours.
3. After incubation, examine tubes for evidence of lysine deaminase, motility and lysine decarboxylase reactions.

Results

Lysine deaminase is indicated by a red or red-brown color in the top centimeter of the medium.

Motility is indicated by a clouding of the medium or by growth extending from the inoculation line.

Lysine decarboxylase is indicated by a purple color throughout the medium. This color may vary in intensity and may be bleached out to a pale light color due to reduction of the indicator. Lysine-negative cultures produce a yellow medium that may be purple or red on the top. Tubes that show a purple reaction with a red color on top should be incubated for a longer period of time.

After examining the medium for lysine deaminase, motility and lysine decarboxylase reactions, add 3 or 4 drops of Indole Reagent Kovacs to the top of each tube. The appearance of a pink to red color in the reagent is interpreted as a positive indole test.

Positive and negative reactions are based on 90% or more occurrences. When an aberrant reaction occurs, subcultures should be plated on differential media to ensure the purity of the culture.

Limitations of the Procedure

1. Do not add Indole Reagent Kovacs until the final lysine deaminase, lysine decarboxylase and motility results have been interpreted.
2. Occasionally, the indole test produces false-negative or falsely weak reactions.[3,4]

References

1. **Reller, L. B., and S. Mirrett.** 1975. Motility-indole-lysine medium for presumptive identification of enteric pathogens of *Enterobacteriaceae.* J. Clin. Microbiol. **2**:247-252.

User Quality Control

Identity Specifications

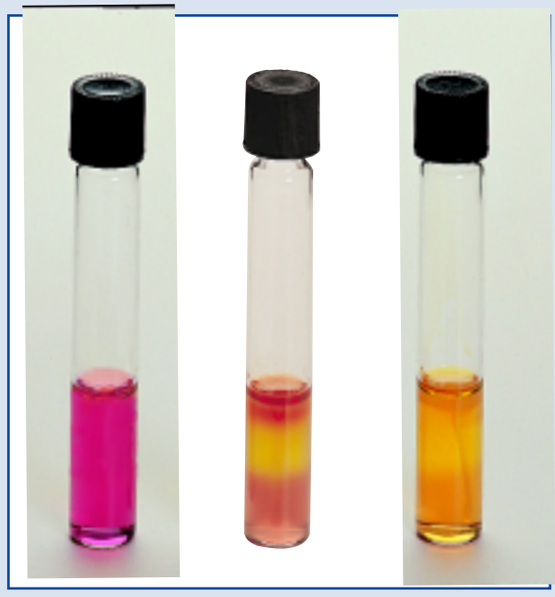
Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.65% solution, soluble in distilled or deionized water upon boiling. Solution is reddish purple, clear.
Prepared Medium:	Reddish purple, clear, semisolid.
Reaction of 3.65% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Prepare MIL Medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After reading the lysine decarboxylase, motility and lysine deaminase reactions, add Indole Reagent Kovacs to determine the indole reaction.

ORGANISM	ATCC®	LYSINE DECARBOXYLASE	MOTILITY	LYSINE DEAMINASE	INDOLE PRODUCTION
Escherichia coli	25922*	+	+	–	+
Providencia alcalifaciens	9886	–	+	+	–
Salmonella enteritidis	13076	+	+	–	–
Shigella flexneri	12022*	–	–	–	–

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



Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Shigella flexneri* ATCC® 12022

All with Indole Reagent

2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
3. **Baron, E. J., and S. M. Finegold.** 1990. Bailey and Scott's Diagnostic Microbiology, 8th ed. The C. V. Mosby Co., St. Louis, MO.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, MD.

Packaging

MIL Medium	500 g	1804-17

Bacto® MIO Medium

Intended Use

Bacto MIO Medium is used for differentiating *Enterobacteriaceae* based on motility, ornithine decarboxylase activity and indole production.

Also Known As

MIO Medium conforms with Motility Indole Ornithine Medium and Ornithine Indole Motility (OIM) Medium.

Summary and Explanation

Tests for indole production, motility and ornithine decarboxylase activity play important roles in the identification of *Enterobacteriaceae.* Ederer and Clark[1] and Oberhofer and Hajkowski[2] developed MIO Medium which combines all three differentiating reactions in onemedium. Ederer and Clark stressed the advantages of MIO Medium in their extensive study comparing cultural reactions of *Enterobacteriaceae* on MIO Medium with reactions on classic media.

Principles of the Procedure

MIO Medium contains peptones which provide carbon and nitrogen. Yeast Extract provides vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon. Dextrose is an energy source. Bacto Agar is added to demonstrate motility. The pH indicator, Brom Cresol Purple, facilitates detection of decarboxylase activity.

Formula

MIO Medium

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Peptone	10 g
Bacto Tryptone	10 g
Bacto L-Ornithine HCl	5 g
Bacto Dextrose	1 g
Bacto Agar	2 g
Bacto Brom Cresol Purple	0.02 g

Final pH 6.5 ± 0.2 at 25°C

User Quality Control

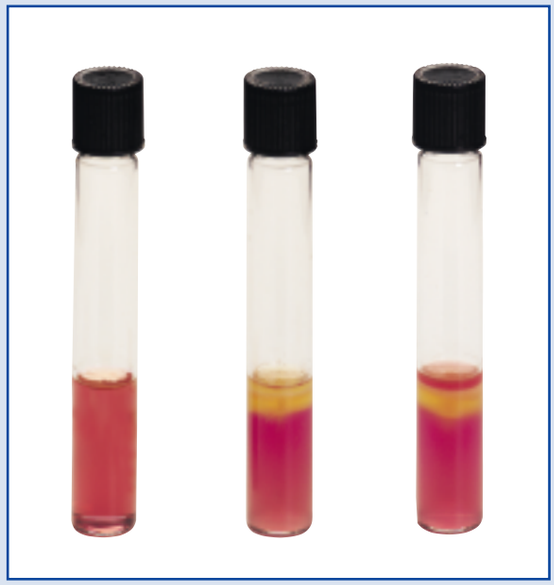
Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in distilled or deionized water upon boiling; purple, clear to slightly opalescent.
Prepared Medium:	Purple, slightly opalescent, semi-solid.
Reaction of 3.1% Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	GROWTH	MOTILITY	INDOLE	ORNITHINE DECARBOXYLASE
Enterobacter aerogenes	13048*	good	+	–	+
Escherichia coli	25922*	good	+	+	+
Klebsiella pneumoniae	13883*	good	–	–	–
Proteus mirabilis	25933	good	+	–	+



Uninoculated tube | *Enterobacter aerogenes* ATCC® 13048 | *Escherichia coli* ATCC® 25922

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.

Refer to appropriate references for typical motility, indole production and ornithine decarboxylase activity of various members of the *Enterobacteriaceae.*[3,4,5,6]

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

MIO Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
SpotTest™ Indole Reagent Kovacs
Inoculating wire

Method of Preparation

1. Suspend 31 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense into test tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Pick isolated colonies with an inoculating wire and stab the medium to the bottom of the tube.
2. Incubate with caps loosened at 35 ± 2°C for 24-48 hours.
3. Examine tubes at 24 hours for growth, color change and motility. A color change from purple to yellow indicates a negative ornithine decarboxylase reaction; no color change indicates a positive reaction. Motility is shown by clouding of the medium or by growth extension from the inoculating line. Repeat the reading at 40-48 hours.
4. Add 3-4 drops of SpotTest™ Indole Reagent Kovacs to each tube. Record as indole positive if a pink or red color appears or as indole negative if there is no color change.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Do not add Kovacs reagent to the tubes until after final motility and ornithine results have been interpreted.
2. To prepare the stored medium for use in motility studies, loosen caps, heat to boiling and cool to 45-50°C prior to inoculation.[5]

References

1. **Ederer, G. M., and M. Clark.** 1970. Motility-Indole-Ornithine medium. Appl. Microbiol. **2**:849.
2. **Oberhofer, T. R., and R. Hajkowski.** 1970. Evaluation of non-lactose-fermenting members of the *Klebsiella-Enterobacter-Serratia* Division. I. Biochemical characteristics. Am. J. Clin. Pathol. **54**:720.
3. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, NY.
4. **Krieg, N. R., and J. G. Holt (ed.).** 1984. Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins, Baltimore, MD.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.).** 1995. Manual of clinical microbiology. 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

MIO Medium	100 g	0735-15
	500 g	0735-17

Bacto® MYP Agar
Bacto Antimicrobic Vial P

Intended Use

Bacto MYP Agar is used with Bacto Egg Yolk Enrichment 50% and Bacto Antimicrobic Vial P for enumerating *Bacillus cereus* from foods.

Also Known As

MYP Agar is also known as Mannitol-Egg Yolk-Polymixin Agar.

Summary and Explanation

Mossel et al[1] formulated Mannitol-Egg Yolk-Polymixin (MYP) Agar to isolate and enumerate *Bacillus cereus* from foods. This medium differentiates *B. cereus* from other bacteria based on its resistance to polymixin, lack of mannitol fermentation, and presence of lecithinase.[2, 3] *B. cereus* is commonly found in nature, on vegetables, and in some processed foods.[4] Under favorable circumstances the microorganism grows to sufficient numbers and causes gastrointestinal illness.[4] Outbreaks of food borne illness have been associated with boiled and cooked rice, cooked meats and cooked vegetables.[5]

MYP Agar is a recommended medium for testing foods.[4, 5, 6]

Principles of the Procedure

MYP Agar contains Beef Extract and Bacto Peptone as sources of carbon, nitrogen, vitamins and minerals. D-Mannitol is the carbohydrate source. Phenol Red is the pH indicator. Bacto Agar is the solidifying agent. Egg Yolk Enrichment 50% provides lecithin. Antimicrobic Vial P is Polymixin B which inhibits the growth of most other bacteria.

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

Formula

MYP Agar

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Peptone	10 g
Bacto D-Mannitol	10 g
Sodium Chloride	10 g
Bacto Phenol Red	0.025 g
Bacto Agar	15 g

Final pH 7.2 ± 0.1 at 25°C

Antimicrobic Vial P

30,000 units polymyxin B

Precautions

1. For Laboratory Use.
2. **Antimicrobic Vial P**: MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) MAY BE HARMFUL IF ABSORBED OR INTRODUCED THROUGH SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

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

Storage

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

Store the desiccated and rehydrated Antimicrobic Vial P 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.

User Quality Control

Identity Specifications

MYP Agar

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 4.6% solution, soluble in distilled or deionized water on boiling. Solution is red, slightly opalescent.

Prepared Medium: Red, slightly opalescent without significant precipitate.

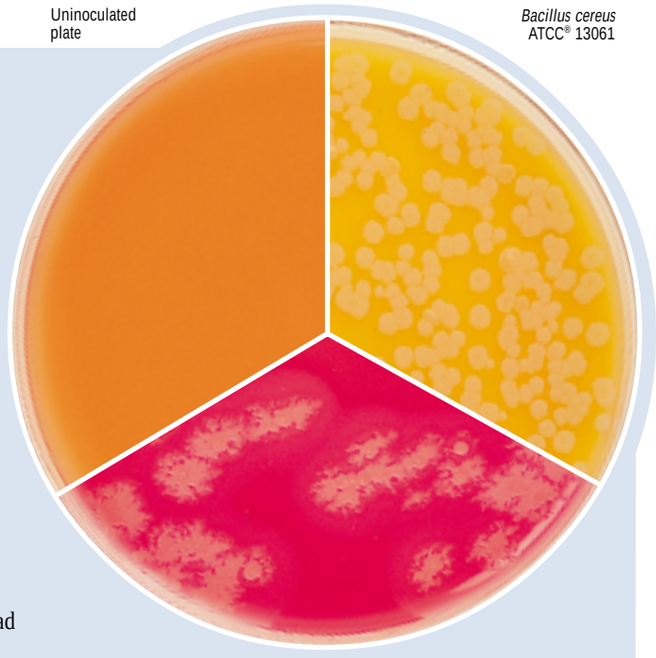
Reaction of 4.6 g/90 ml distilled or deionized water at 25°C: pH 7.2 ± 0.1

Antimicrobic Vial P

Dehydrated Appearance: White cake or powder.

Cultural Response

Prepare MYP Agar per label directions. Supplement with Egg Yolk Enrichment 50% and Antimicrobic Vial P (Polymyxin B). Inoculate and incubate at 30 ± 2°C for 18-48 hours. Lecithinase reaction is read as a zone of precipitate. Colonies that ferment mannitol are yellow.



ORGANISM	ATCC®	INOCULUM CFU	GROWTH	MANNITOL FERMENTATION	LECITHINASE REACTION
Bacillus cereus	13061	3-30 and 30-300	good	–	+
Bacillus subtilis	6633	3-30 and 30-300	good	+	–
Pseudomonas aeruginosa	27853*	1,000-2,000	inhibited	–	–

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

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

Procedure

Materials Provided

MYP Agar
Egg Yolk Enrichment 50%
Antimicrobic Vial P

Materials Required but not Provided

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

Method of Preparation

MYP Agar

1. Suspend 46 grams of MYP Agar in 900 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 225 ml into 500 ml flasks.
4. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
5. Aseptically add 12.5 ml Egg Yolk Enrichment 50% and 4.1 ml rehydrated Antimicrobic Vial P (25,000 units of polymyxin B). Mix thoroughly.

Antimicrobic Vial P

1. Rehydrate with 5 ml sterile water.

Specimen Collection and Preparation

Consult appropriate references.[4,5,6]

Test Procedure

Consult appropriate references.[4,5,6]

Results

Consult appropriate references.[4,5,6]

References

1. **Mossel, D. A. A., M. J. Koopman, and E. Jongerius.** 1967. Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. **15**:650-653.
2. **Donovan, K. O.** 1958. A selective medium for *Bacillus cereus* in milk. J. Appl. Bacteriol. **21**:100-103.
3. **Coliner, A. R.** 1948. The action of *Bacillus cereus* and related species on the lechithin complex of egg yolk. J. Bacteriol. **55**:777-785.
4. **Jeffery, E. J., and S. M. Harmon.** 1995. *Bacillus cereus*, p. 14. 01-14.08. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Harmon, S. M., J. M. Goepfert, and R. W. Bennett.** 1992. *Bacillus cereus*, p. 593-604. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.

Packaging

MYP Agar	500 g	0810-17
Antimicrobial Vial P	6 x 10 ml	3268-60*

* Store at 2-8°C

Bacto® MacConkey Broth

User Quality Control

Identity Specifications

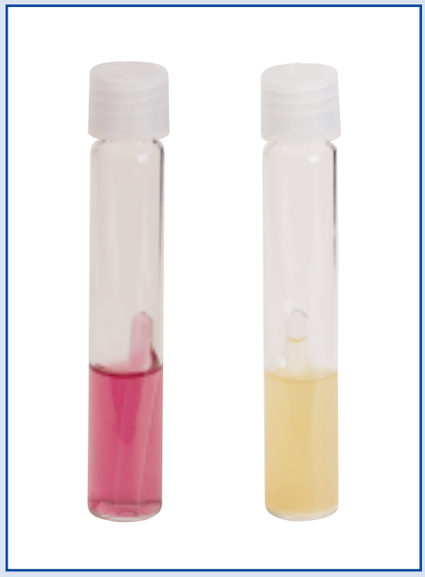
Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water; purple, clear.
Prepared Tubes:	Purple, clear.
Reaction of 3.5% Solution at 25°C:	pH 7.3 ± 0.1

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	MEDIUM COLOR	GAS
Enterococcus faecalis	29212*	1,000-2,000	markedly inhibited	purple	–
Escherichia coli	25922*	100-1,000	good	yellow	+

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



Uninoculated tube — *Escherichia coli* ATCC® 25922

Intended Use

Bacto MacConkey Broth is used for cultivating gram-negative, lactose-fermenting bacilli in water and foods as a presumptive test for coliform organisms.

Summary and Explanation

MacConkey Broth is a modification of the original bile salt broth recommended by MacConkey[1] that contained 0.5% sodium taurocholate and litmus as an indicator. In later publications,[2,3] MacConkey suggested variations of this formulation using neutral red indicator instead of litmus. Childs and Allen[4] demonstrated the inhibitory effect of neutral red and substituted the less inhibitory brom cresol purple. Oxgall in the medium replaces the original sodium taurocholates to inhibit growth of gram-positive organisms.

Principles of the Procedure

Peptone provides amino acids and other growth factors. Lactose is a carbon energy source for gram-negative lactose-fermenting bacilli. Oxgall inhibits the growth of gram-positive organisms. Brom Cresol Purple is the indicator.

Formula

MacConkey Broth

Formula Per Liter

Bacto Oxgall	5 g
Bacto Peptone	20 g
Bacto Lactose	10 g
Bacto Brom Cresol Purple	0.01 g

Final pH 7.3 ± 0.1 at 25°C

Precautions

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

   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 MacConkey Broth below 30°C. The powder is very hygoscopic. 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

MacConkey Broth

Materials Provided But Not Required

Glassware
Autoclave
Incubator (35°C)
Tubes with closures

Method of Preparation

1. Dissolve 35 grams in 1 liter of distilled or deionized water. Rehydrate with proportionally less water when liquid inocula will exceed 1 ml.
2. Dispense into tubes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines.
2. Process each specimen as appropriate for that specimen.
3. Inoculate specimen as appropriate for that specimen.
4. Incubate tubes for 18-24 hours at 35 ± 2°C.
5. Examine tubes.

Test Procedure

See appropriate references for specific procedures.

Results

Lactose-fermenting organisms grow very well in MacConkey Broth and produce acid, causing the medium to turn yellow. Gas is also produced. Non-fermenting organisms produce good growth but will not produce acid or gas.

References

1. **MacConkey, A.** 1901. Centr. Bakt. **29**:740.
2. **MacConkey, A.** 1905. Lactose-fermenting bacteria in faeces. J. Hyg. **5**:333-379.
3. **MacConkey, A.** 1908. Bile salt media and their advantage in some bateriological examinations. J. Hyg. **8**:322-334.
4. **Childs, E., and L. A. Allen.** 1953. Improved methods for determining the most probable number of *Bacterium coli* and of *Streptococcus faecalis*. J. Hyg. Camb. **51**:468-477.

Packaging

MacConkey Broth	500 g	0020-17

MacConkey Media

Bacto® MacConkey Agar · Bacto MacConkey Agar Base
Bacto MacConkey Agar CS · Bacto MacConkey Agar w/o CV
Bacto MacConkey Agar w/o Salt

Intended Use

MacConkey Media are selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical,[1] dairy,[2] food,[3,4] water,[5] pharmaceutical[6] and industrial[7] sources.

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

Bacto MacConkey Agar Base is used with added carbohydrate in differentiating coliforms based on fermentation reactions.

User Quality Control

Identity Specifications

MacConkey Agar

Dehydrated Appearance:	Pink to pinkish beige, free-flowing, homogenous.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling; reddish purple, very slightly to slightly opalescent.
Prepared Plates:	Pinkish red, slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

MacConkey Agar Base

Dehydrated Appearance:	Pink to pinkish beige, free-flowing, homogenous.
Solution:	4.0% solution, soluble in distilled or deionized water upon boiling; red, very slightly to slightly opalescent without significant precipitate.
Prepared Plates:	Red, slightly opalescent without precipitate.
Reaction of 4.0% Solution at 25°C:	pH 7.1 ± 0.2

MacConkey Agar CS

Dehydrated Appearance:	Pinkish beige, homogenous, free-flowing.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling; reddish purple in color, slightly opalescent, without significant precipitate.
Prepared Plates:	Reddish purple, slightly opalescent, without precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

continued on following page

Bacto MacConkey Agar CS is used for isolating and differentiating gram-negative enteric bacilli from specimens containing swarming strains of *Proteus.*

Bacto MacConkey Agar w/o CV is used for isolating and differentiating enteric microorganisms while permitting growth of staphylococci and enterococci.

Bacto MacConkey Agar w/o Salt is used for isolating and differentiating gram-negative bacilli while suppressing the swarming of most *Proteus* species.

Also Known As

MacConkey Agar is also known as MAC.

Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.[8]

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved the growth of *Shigella* and *Salmonella* strains. These modifications included the addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations. The formula improvements gave improved differential reactions between these enteric pathogens and the coliform group.

MacConkey Agar contains crystal violet and bile salts that inhibit gram-positive organisms and allow gram-negative organisms to grow. Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation. Colonies that do not ferment lactose (such as typhoid, paratyphoid and dysentery bacilli) remain colorless. When lactose non-fermenters grow in proximity to coliform colonies, the surrounding medium appears as cleared areas.

MacConkey Agar Base is prepared without added carbohydrates, which permits their addition either individually or in combination. It is recommended that carbohydrates such as sucrose or lactose be added in a concentration of 1% to the basal medium.

MacConkey CS ("Controlled Swarming") contains carefully selected raw materials to reduce the swarming of *Proteus* species which could cause difficulty in isolating and enumerating other gram-negative bacilli.

MacConkey Agar w/o CV (Crystal Violet) is a differential medium that is less selective than MacConkey Agar. The lack of crystal violet permits the growth of *Staphylococcus* and *Enterococcus.* Staphylococci produce pale pink to red colonies and enterococci produce compact tiny red colonies either on or beneath the surface of the medium.

MacConkey Agar w/o Salt is a differential medium that restricts the swarming of *Proteus* species to aid in the detection and isolation of enteric microorganisms. In addition, this medium does not contain crystal violet, allowing *Staphyloccocus* and *Enterococcus* species to grow.

Principles of the Procedure

Bacto Peptone and Proteose Peptone are sources of nitrogen and other nutrients. Lactose is a fermentable carbohydrate. When lactose is fermented, a local pH drop around the colony causes a color change in the pH indicator (neutral red) and bile precipitation. Bile Salts, Bile Salts No. 3 and Crystal Violet are selective agents that inhibit growth of gram-positive organisms. Bacto Agar is a solidifying agent.

Formula

MacConkey Agar

Formula Per Liter

Bacto Peptone ..... 17 g
Bacto Proteose Peptone ..... 3 g
Bacto Lactose ..... 10 g
Bacto Bile Salts, No. 3 ..... 1.5 g
Sodium Chloride ..... 5 g
Bacto Agar ..... 13.5 g
Neutral Red ..... 0.03 g
Bacto Crystal Violet ..... 0.001 g

Final pH 7.1 ± 0.2 at 25°C

MacConkey Agar Base

Formula Per Liter

Bacto Peptone ..... 17 g
Bacto Proteose Peptone ..... 3 g
Bacto Bile Salts, No. 3 ..... 1.5 g
Sodium Chloride ..... 5 g
Bacto Agar ..... 13.5 g
Neutral Red ..... 0.03 g
Bacto Crystal Violet ..... 0.001 g

Final pH 7.1 ± 0.2 at 25°C

MacConkey Agar CS

Formula Per Liter

Bacto Peptone ..... 17 g
Bacto Proteose Peptone ..... 3 g
Bacto Lactose ..... 10 g
Bacto Bile Salts ..... 5 g
Sodium Chloride ..... 5 g
Bacto Agar ..... 13.5 g
Neutral Red ..... 0.03 g
Bacto Crystal Violet ..... 0.001 g

Final pH 7.1 ± 0.2 at 25°C

MacConkey Agar w/o CV

Formula Per Liter

Bacto Peptone ..... 20 g
Bacto Lactose ..... 10 g
Bacto Bile Salts ..... 5 g
Sodium Chloride ..... 5 g
Neutral Red ..... 0.05 g
Bacto Agar ..... 12 g

Final pH 7.4 ± 0.2 at 25°C

MacConkey Agar w/o Salt

Formula Per Liter

Bacto Peptone ..... 20 g
Bacto Lactose ..... 10 g
Bacto Bile Salts ..... 5 g
Neutral Red ..... 0.075 g
Bacto Agar ..... 12 g

Final pH 7.4 ± 0.2 at 25°C

User Quality Control cont.

MacConkey Agar w/o CV

Dehydrated Appearance:	Pinkish beige, free-flowing, homogenous.
Solution:	5.2% solution, soluble in distilled or deionized water upon boiling; reddish orange, clear to very slightly opalescent without significant precipitate.
Prepared Plates:	Reddish orange, slightly opalescent without significant precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

MacConkey Agar w/o Salt

Dehydrated Appearance:	Pinkish beige, free-flowing, homogenous.
Solution:	4.7% solution, soluble in distilled or deionized water upon boiling; reddish orange, slightly opalescent.
Prepared Plates:	Reddish orange, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

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

MacConkey Agar

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	1,000-2,000	markedly to completely inhibited	–	–
Escherichia coli	25922*	100-1,000	good	pink	+
Proteus mirabilis	12453	100-1,000	good	colorless	–
Salmonella typhimurium	14028*	100-1,000	good	colorless	–

continued on following page

Precautions

1. For Laboratory Use.
2. **For MacConkey Agar w/o CV**
   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.

MacConkey Agar w/o Salt

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

User Quality Control cont.

MacConkey Agar Base

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	1,000-2,000	markedly to completely inhibited	–	–
Escherichia coli	25922*	100-1,000	good	w/o lactose: colorless w/lactose: pink	+ +
Proteus mirabilis	12453	100-1,000	good	colorless	–
Salmonella typhimurium	14028*	100-1,000	good	colorless	–

MacConkey Agar CS

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	1,000-2,000	markedly to completely inhibited	–	–
Escherichia coli	25922*	100-1,000	good	pink to red	–/+
Proteus mirabilis	12453	100-1,000	good	colorless, swarming markedly to completely inhibited	–
Salmonella typhimurium	14028*	100-1,000	good	translucent, colorless	–

MacConkey Agar w/o CV

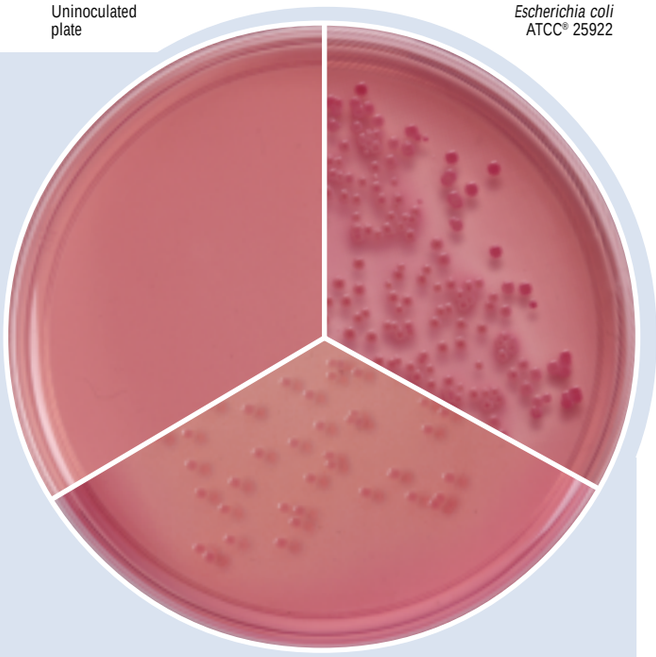
ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	100-1,000	good	red	–
Escherichia coli	25922*	100-1,000	good	pink or red	–
Proteus mirabilis	12453	100-1,000	good	colorless	–
Salmonella typhimurium	14028*	100-1,000	good	colorless	–

MacConkey Agar w/o Salt

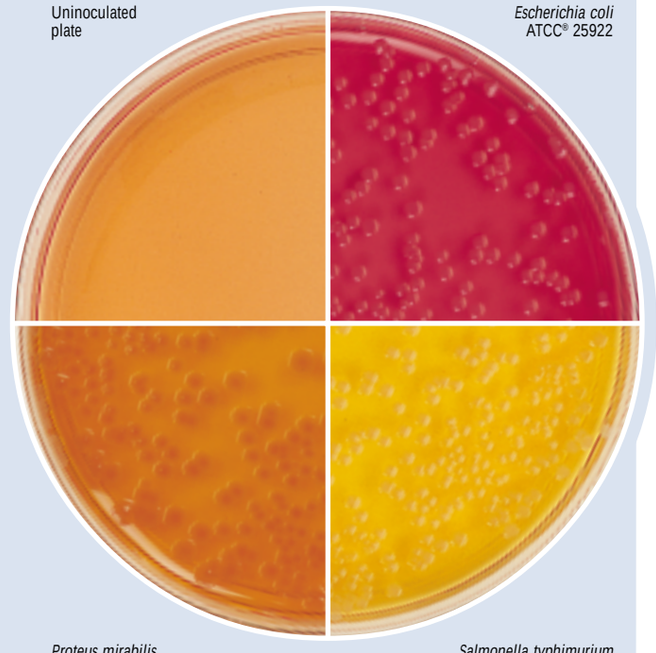
ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	100-1,000	good	red	–
Escherichia coli	25922*	100-1,000	good	pink to red	–
Proteus mirabilis	12453	100-1,000	good	colorless; swarming markedly to completely inhibited	– –
Salmonella typhimurium	14028*	100-1,000	good	colorless	–

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

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



MacConkey Agar CS



MacConkey Agar w/o CV

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

MacConkey Agar
MacConkey Agar Base
MacConkey Agar CS
MacConkey Agar w/o CV
MacConkey Agar w/o Salt

Materials Required But Not Provided

Glassware
Autoclave
35°C incubator
50°C waterbath (optional)
Carbohydrate (lactose, sucrose, etc.) (optional)

Method of Preparation

For MacConkey Agar, MacConkey Agar CS, MacConkey Agar w/o CV or MacConkey Agar w/o Salt:

1. Suspend the medium in 1 liter distilled or deionized water:

MacConkey Agar	50 grams
MacConkey Agar CS	50 grams
MacConkey Agar w/o CV	52 grams
MacConkey Agar w/o Salt	47 grams

2. Heat to boiling to dissolve completely. Avoid overheating.
3. Autoclave at 121°C for 15 minutes. The media may be used without autoclave sterilization if the plates are to be inoculated on the day of preparation.
4. Cool to 45-50°C and dispense into sterile Petri dishes.
5. The surface of the medium should be dry when inoculated. Dry the plates for 1-2 hours with the lids slightly ajar.

For MacConkey Agar Base:

1. Suspend 40 grams of medium in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Avoid overheating.
3. Add 10 grams lactose or other desired carbohydrate before or after sterilization, depending on heat lability.
4. Autoclave at 121°C for 15 minutes. The media may be used without autoclave sterilization if the plates are to be inoculated on the day of preparation. In this case, boiling the medium gently for 5 minutes is sufficient.
5. Cool to 45-50°C and dispense into sterile Petri dishes.
6. The surface of the medium should be dry when inoculated. Dry the plates for 1-2 hours with the lids slightly ajar.

Specimen Collection and Preparation

For a complete discussion on the isolation and identification of enteric organisms consult the appropriate references.

Test Procedure

For procedures on the isolation and identification of enteric organisms consult the appropriate references.

Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or with out a zone of precipitated bile. Non-lactose fermenting organisms grow as colorless or clear colonies.

Swarming by *Proteus* spp. is reduced on MacConkey Agar CS and MacConkey Agar w/o Salt.

On MacConkey Agar w/o CV and MacConkey Agar w/o Salt, staphylococci produce pale pink to red colonies and enterococci produce tiny red colonies; these organisms are inhibited on MacConkey Agar and MacConkey Agar CS.

Limitations of the Procedure

1. Although MacConkey media are selective primarily for gram-negative enteric bacilli, biochemical and, if indicated, serological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[1,3]
2. Due to the selective properties of MacConkey Agar CS, some strains of gram-negative enteric bacilli may be encountered that fail to grow or grow poorly on this medium. Some strains of gram-positive organisms may be encountered that are not inhibited or only partially inhibited on this medium; some strains of enterococci may grow on MacConkey Agar CS after prolonged incubation.
3. Incubation of MacConkey Agar plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.[9]
4. For optimal performance, plates prepared from MacConkey Agar CS should be incubated under aerobic conditions.

References

1. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*, p. 450-456. *In* P. R. Murray, E. J. Baron, M. A, Pfaller, F. C. Tenover, and R. H Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig (H.M. Wehr, Tech. Comm.).** 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.

3. **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.
4. **Food and Drug Administration**. 1995. Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, MD.
5. **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.
6. **United States Pharmacopeial Convention, Inc.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
7. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
8. **MacConkey, A.** 1905. Lactose-fermenting bacteria in feces. J. Hyg. **5:**333-379.
9. **Mazura-Reetz, G. T. Neblett, and J. M. Galperin**. 1979. MacConkey Agar: CO_2 vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

Packaging

MacConkey Agar	100 g	0075-15-3
	500 g	0075-17-1
	2 kg	0075-07-3
	10 kg	0075-08-2
MacConkey Agar Base	500 g	0818-17-3
MacConkey Agar CS	500 g	1818-17-1
	2 kg	1818-07-3
	10 kg	1818-08-2
MacConkey Agar w/o CV	500 g	0470-17-2
MacConkey Agar w/o Salt	500 g	0331-17-1
	10 kg	0331-08-2

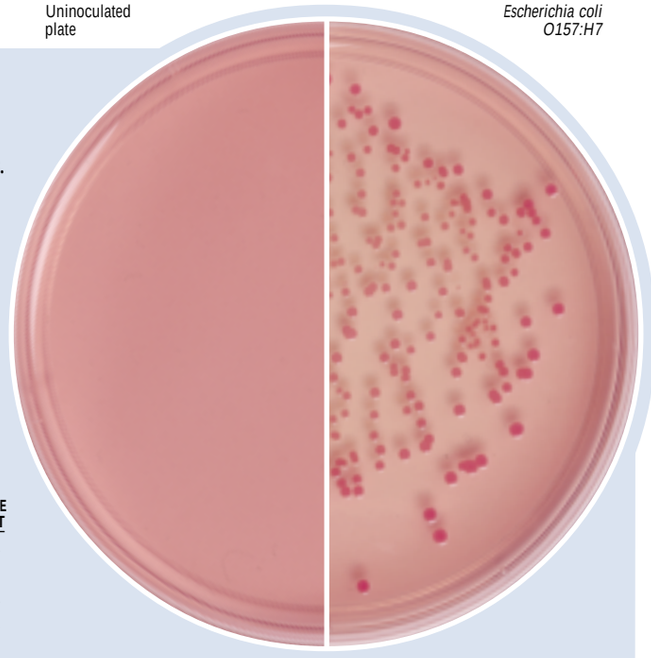
Bacto® MacConkey Sorbitol Agar

Intended Use

Bacto MacConkey Sorbitol Agar is used for isolating and differentiating enteropathogenic *Escherichia coli* serotypes.

Summary and Explanation

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications used in MacConkey Agar improved the growth of *Shigella* and *Salmonella* strains as well as the differential reactions between these enteric pathogens and the coliform group. The modifications included addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations.



User Quality Control

Identity Specifications

Dehydrated Appearance:	pinkish-beige, free flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling; reddish purple, very slightly to slightly opalescent.
Prepared Medium:	reddish-purple, slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2 at 25°C

Cultural Response

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

ORGANISM	ATCC®	CFU	INOCULUM RECOVERY	COLONY COLOR	BILE PPT
Enterococcus faecalis	29212*	1,000-2,000	markedly inhibited	–	–
Escherichia coli 0157:H7	–	100-1,000	good	colorless	–
Escherichia coli	25922*	100-1,000	good	pink-red	+

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.

MacConkey Sorbitol Agar is a modification of the formula given by Rappaport and Henig[1] for isolating enteropathogenic *Escherichia coli* serotypes 011 and 055. The usefulness of this medium in detecting *E. coli* 0157:H7, a human pathogen associated with hemorrhagic colitis, has been described.[2,3,4]

This medium employs d-sorbitol rather than lactose for isolating and differentiating the enteropathogenic *E. coli* serotypes which tend to be sorbitol negative. This medium can be used for clinical and food testing.[1,5,6]

Principles of the Procedure

Bacto Peptone and Proteose Peptone are nitrogen sources in the medium. D-Sorbitol is a fermentable carbohydrate. Many hemorrhagic *E. coli* strains will not ferment d-sorbitol and appear as colorless colonies on MacConkey Sorbitol Agar. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is a pH indicator. Bacto Agar is a gelling agent.

Formula

MacConkey Sorbitol Agar

Formula Per Liter

Bacto Peptone	15.5 g
Bacto Proteose Peptone	3 g
d-Sorbitol	10 g
Bacto Bile Salts	1.5 g
Sodium Chloride	5 g
Bacto Agar	15 g
Neutral Red	0.03 g
Bacto Crystal Violet	0.001 g

Final pH 7.1 ± 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

MacConkey Sorbitol Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 50 grams in 1 liter distilled or deionized water:
2. Heat to boiling to dissolve completely. Avoid overheating.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes.
5. Dry plates for 1-2 hours with the lids slightly ajar. The surface of the medium should be dry when inoculated.

MacConkey Sorbitol Agar may be used without autoclave sterilization if the plates are to be used on the day of preparation. Boil the medium 2-3 minutes before pouring into Petri dishes and dry before inoculation.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and immediately transport to the laboratory in accordance with recommended guidelines.
2. Process each specimen as appropriate for that specimen.
3. Inoculate the specimen onto medium appropriate for that specimen.
4. Incubate plates for 18-24 hours at 35 ± 2°C.
5. Examine plates.

Test Procedure

See appropriate references for specific procedures.

Results

Sorbitol-fermenting organisms produce pink colonies on MacConkey Sorbitol Agar. Organisms that do not ferment sorbitol, such as *E. coli* 0157:H7, are colorless.

Limitations of the Procedure

1. The color of sorbitol-positive colonies can fade, making them hard to distinguish from sorbitol-negative colonies.[3]
2. Upon prolonged incubation, strains of *E. coli* 0157:H7 can ferment sorbitol.[3]
3. Strains of other organisms that do not ferment sorbitol may grow on MacConkey Sorbitol Agar. It is necessary to select suspected colonies for further identification.[3]
4. The sole use of this medium can cause the microbiologist to miss other organisms that may be pathogenic.[7]
5. To isolate *E. coli* 0157:H7 from clinical specimens, inoculate fecal specimens and rectal swabs on a small area of one quadrant and streak for isolation. This will permit development of discrete colonies.

References

1. **Rappaport, F., and E. Henig**. 1952. Media for the isolation and differentiation of pathogenic *Escherichia coli* (serotypes 0111 and 055). J. Clin. Pathology. **5:**361-362.
2. **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. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Adams, S**. 1991. Screening for verotoxin-producing *Escherichia coli*. Clinical Lab Science **4(1):**19-20.
4. **March, S. B., and S. Ratnam**. 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* 0157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **23:**869-872.
5. **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.

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. **Ewing, W. H., and P. R. Edwards**. 1954. Isolation and preliminary identification of *Escherichia coli* serotypes associated with cases of diarrhea of the newborn. Public Health Lab. **12:**75-81.

Packaging

MacConkey Sorbitol Agar	500 g	0079-17

Bacto® Malonate Broth

Intended Use

Bacto Malonate Broth is used for differentiating *Enterobacter* from *Escherichia* based on malonate utilization.

Summary and Explanation

Malonate Broth, prepared according to the formula described by Leifson[1], is a liquid medium containing ammonium sulfate as the only source of nitrogen and malonate as the only source of carbon. Leifson was able to demonstrate that the *Enterobacter* group utilizes malonate whereas the *Escherichia* group is unable to grow on the medium.

Malonate Broth is further described for differentiating *Enterobacteriaceae* in food and dairy products.[2,3,4] In some cases, however, the medium referenced is the modified Edwards and Ewing[5] formulation that contains yeast extract and dextrose. The modification permits growth of organisms that would otherwise fail on the Leifson medium.

Principles of the Procedure

Malonate Broth contains Ammonium Sulfate, which is the sole source of nitrogen in the medium; Sodium Malonate is the sole source of carbon. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capability. Sodium Chloride maintains the osmotic balance of the medium. Increased alkalinity resulting from malonate utilization causes the indicator, Brom Thymol Blue, to change color from green to blue.

Formula

Malonate Broth

Formula Per Liter

Ammonium Sulfate	2 g
Dipotassium Phosphate	0.6 g
Monopotassium Phosphate	0.4 g
Sodium Chloride	2 g
Sodium Malonate	3 g
Bacto Brom Thymol Blue	0.025 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. 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.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light green, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in distilled or deionized water. Solution is green, clear.
Reaction of 0.8% Solution at 25°C:	pH 6.7 ± 0.2

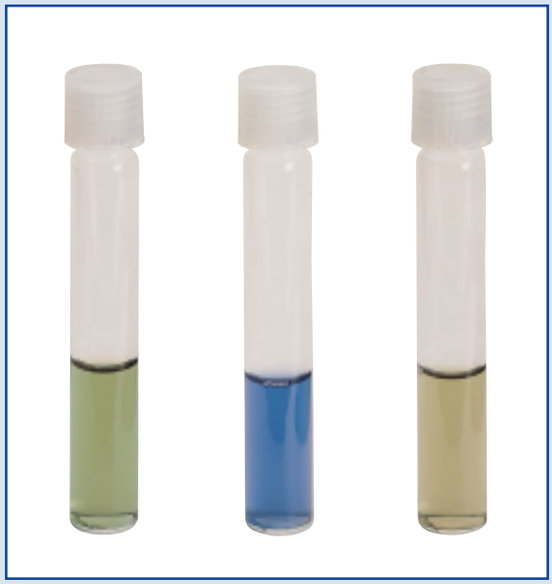
Cultural Response

Prepare Malonate Broth per label directions. Inoculate the medium with a loopful of test organism and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	APPEARANCE
Enterobacter aerogenes	13048*	good	blue
Escherichia coli	25922*	poor to fair	green

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 | *Enterobacter aerogenes* ATCC® 13048 | *Escherichia coli* ATCC® 25922

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

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

Procedure
Materials Provided
Malonate Broth

Materials Required But Not Provided
Glassware
Autoclave
Incubator (35°C)

Method of Preparation
1. Dissolve 8 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Avoid introducing extraneous carbon and nitrogen.

Specimen Collection
Refer to appropriate references for specimen collection and preparation.

Test Procedure
1. Inoculate tubes with a loopful of test organism.
2. Incubate at 35 ± 2°C for 18-48 hours.
3. Examine tubes for a change in the color of the medium from green to blue.

Results
Malonate utilization is indicated by a change in the color of the medium from green to blue:
Positive: Blue
Negative: Green

Limitations of the Procedure
1. A slight bluing (blue-green) of the medium may occur after prolonged incubation.[6] In such cases, care should be taken in interpreting results.

References

1. **Leifson, E.** 1933. The fermentation of sodium malonate as a means of differentiating *Aerobacter* and *Escherichia*. J. Bacteriol. **26**: 329.
2. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International. Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Edwards, P. R., and W. H. Ewing**. 1962. *Enterobacteriaceae.* U.S. Public Health Service Bulletin No. **734**:19.
6. **Oberhofer, T. R.** 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, NY.

Packaging
Malonate Broth	100 g	0395-15
	500 g	0395-17

Bacto® Malonate Broth Modified

User Quality Control
Identity Specifications
Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	0.93% solution, soluble in distilled or deionized water with agitation. Solution is green, clear.
Reaction of 0.93% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response
Prepare Malonate Broth Modified per label directions. Inoculate the medium with a loopful of undiluted organism and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM	COLOR OF MEDIUM
Enterobacter aerogenes	13048*	undiluted	blue
Escherichia coli	25922*	undiluted	green
Salmonella arizonae	13314	undiluted	blue
Salmonella typhimurium	14028*	undiluted	green

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.

Intended Use
Bacto Malonate Broth Modified is used for differentiating *Enterobacteriaceae* based on malonate utilization.

Also Known As
Malonate Broth Modified conforms with Malonate Broth, Ewing.

Summary and Explanation
Malonate Broth Modified is essentially Malonate Broth to which Yeast Extract and Dextrose have been added.[1] These additional ingredients initiate growth of some organisms that otherwise would fail to grow on the unmodified medium and, thus, permit observation of those organisms' malonate activity.

Malonate utilization by microorganisms is indicated by an increase in alkalinity and development of a deep blue color in the medium. Malonate utilization forms a basis on which organisms can be differentiated when testing food products for *Enterobacteriaceae.*[2,3,4] It is useful in the differentiation of *Escherichia coli* from the *Klebsiella-Enterobacter* groups and is considered especially valuable in the differentiation of *Salmonella*. The majority of salmonellae do not utilize malonate whereas *Salmonella arizonae* does.[4,5,6]

This medium may be used in conjunction with phenylalanine as proposed by Shaw and Clarke[7] to detect both malonate degradation and phenylalanine deamination.

Principles of the Procedure

Yeast Extract and Dextrose provide the vitamins and cofactors, as well as minimal sources of carbon, required for good growth of a wide variety of organisms. An organism that can utilize Sodium Malonate as its carbon source while at the same time using Ammonium Sulfate as its nitrogen source causes increased alkalinity due to the formation of sodium hydroxide. This causes the indicator, Brom Thymol Blue, to change color from green to blue. Some malonate-negative strains produce a yellow color. This is due to fermentation of the dextrose alone, producing increased acidity that causes the indicator to turn yellow at pH 6.0. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capability. Sodium Chloride maintains the osmotic balance of the medium.

Formula

Malonate Broth Modified

Formula Per Liter	
Bacto Yeast Extract	1 g
Ammonium Sulfate	2 g
Dipotassium Phosphate	0.6 g
Monopotassium Phosphate	0.4 g
Sodium Chloride	2 g
Sodium Malonate	3 g
Bacto Dextrose	0.25 g
Bacto Brom Thymol Blue	0.025 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

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

Malonate Broth Modified

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 9.3 grams in 1 liter distilled or deionized water and agitate to dissolve completely.
2. Dispense into tubes.
3. 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

A color change of the medium to blue indicates that malonate has been utilized.

Limitations of the Procedure

1. Some malonate-positive organisms produce only slight alkalinity. Compare any tube in question with an uninoculated malonate tube. Any trace of blue color after a 48-hour incubation period denotes a positive test. Before making a final negative interpretation, be sure that test tubes have been incubated for 48 hours.[7]

References

1. **Edwards, P. R. and W. H. Ewing.** 1962. *Enterobacteriaceae.* U.S. Public Health Service Bulletin No. **734**:19.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.
4. **Bacteriological Analytical Manual, 8th ed.** 1995. AOAC International, Gaithersburg, MD.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology. Washington, D.C.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Malonate Broth Modified	500 g	0569-17

Bacto® Malt Agar

Intended Use

Bacto Malt Agar is used for isolating and cultivating yeasts and molds from food, and for cultivating yeast and mold stock cultures.

Summary And Explanation

Malt media for yeasts and molds have been widely used for many years. In 1919, Reddish[1] prepared a satisfactory substitute for beer wort from malt extract. Thom and Church[2] used Reddish's medium for their studies of the aspergilli. Malt Agar was also employed by Fullmer and Grimes[3] for their studies of the growth of yeasts on synthetic media. Malt Agar is specified in Standard Methods[4,5] for the examination of yeasts and molds.

Principles Of The Procedure

Malt Agar contains Malt Extract which provides the carbon, protein and nutrient sources required for the growth of microorganisms. Bacto Agar is a solidifying agent. The acidic pH of Malt Agar allows for optimal growth of molds and yeasts while restricting bacterial growth.

Formula

Malt Agar

Formula per liter

Bacto Malt Extract	30 g
Bacto Agar	15 g

Final pH 5.5 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Malt Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator

Method of Preparation

1. Suspend 45 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. To alter the medium to pH 4.5 or pH 3.5, add the quantity of sterile 85% lactic acid USP indicated on the label. (This quantity is specific to the lot of product.) Do not reheat the medium after adding acid.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

User Quality Control

Identity Specifications

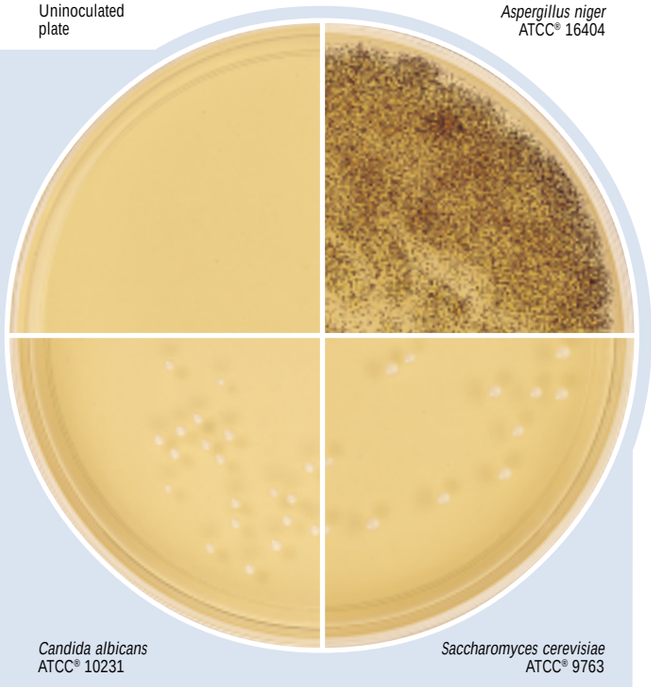
Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.5% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber in color, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, very slightly to slightly opalescent.
Reaction of a 4.5% Solution at 25°C:	5.5 ± 0.2

Cultural Response

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

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

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



Test Procedure
See appropriate references for specific procedures.

Results
Refer to appropriate references and procedures for results.

Limitations of the Procedure
1. Do not heat the medium after addition of acid, as this will hydrolyze the agar and reduce its solidifying properties.

References

1. Abs. Bact., **3**:6, 1919.
2. **Thom, C., and M. B. Church.** 1926. The Aspergilli. Williams and Wilkins Co., Baltimore, MD.
3. **Fulmer, E. I., and M. J. Grimes.** 1923. The growth of yeasts on synthetic agar media. Bacteriol., **8**:585-588.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Agricultural Chemists.** 1995. Official methods of analysis, 16th ed. Association of Official Agricultural Chemists, Washington, D.C.

Packaging
Malt Agar	500 g	0024-17
	10 kg	0024-08

Bacto® Malt Extract

Intended Use
Bacto Malt Extract is used for preparing microbiological culture media for the propagation of yeasts and molds.

Summary and Explanation
Malt Extract is obtained from barley. It is a useful ingredient of culture media designed for the propagation of yeasts and molds. This product is particularly suitable for yeasts and molds because it contains a high concentration of carbohydrates, particularly maltose. The approximate percentage of reducing sugars in Malt Extract is 60-63%. Malt Extract is generally employed in culture media at concentrations between 10 to 100 grams per liter.

Malt Agar, a medium recommended for the detection and isolation of yeasts and molds from dairy products, food and as a stock culture contains Malt Extract. Wort Agar, used for the cultivation and enumeration of yeasts, has Malt Extract as one of the main ingredients in the formula. Several media containing Malt Extract are specified in standard methods.[1,2,3]

User Quality Control
Identity Specifications
Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	2% solution, soluble in distilled or deionized water; medium amber, slightly opalescent to opalescent, may have a precipitate.
Reaction of 2% Solution at 25°C:	pH 4.5 -5.5

Cultural Response
Prepare 2% solution. Inoculate tubes with the test organisms. Incubate tubes at 30 ± 2°C for up to three days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good

The cultures listed are the minimum that should be used.

Principles of the Procedure
Malt Extract provides carbon, protein and nutrients for the isolation and cultivation of yeasts and molds in bacterial culture media.

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

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

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

Procedure
Materials Provided
Malt Extract

Materials Required But Not Provided
Materials vary depending on the medium being prepared.

Method of Preparation
Refer to the final concentration of Malt Extract in the formula of the medium being prepared. Add Malt Extract 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 Malt Extract.

Results
Refer to appropriate references and procedures for results.

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Malt Extract	100 g	0186-15
	500 g	0186-17
	10 kg	0186-08

Bacto® Malt Extract Agar
Bacto® Malt Extract Broth

Intended Use

Bacto Malt Extract Agar is used for isolating, cultivating and enumerating yeasts and molds.

Bacto Malt Extract Broth is used for cultivating yeasts and molds.

User Quality Control

Identity Specifications

Malt Extract Agar

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	3.36% solution, soluble in distilled or deionized water on boiling. Solution is very light amber, slightly opalescent.
Prepared Medium:	Very light amber, slightly opalescent.
Reaction of 3.36% Solution at 25°C:	pH 4.7 ± 0.2

Malt Extract Broth

Dehydrated Appearance:	Light beige to beige, free-flowing, homogeneous.
Solution:	1.5% solution, soluble in distilled or deionized water. Solution is very light amber to light amber, clear without significant precipitate.
Prepared Medium:	Light amber, clear without significant precipitate.
Reaction of 1.5% Solution at 25°C:	pH 4.7 ± 0.2

Cultural Response

Prepare Malt Extract Agar or Malt Extract Broth per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

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

Summary And Explanation

The use of malt and malt extracts for the propagation of yeasts and molds is quite common. Reddish[1] described a culture medium prepared from malt extract that was a satisfactory substitute for wort. Thom and Church,[2] following the formula of Reddish, used Malt Extract as a base from which they prepared the complete media. Malt Extract Broth is specified in standard methods for the examination of yeasts and molds.[3,4]

Principles Of the Procedure

Malt Extract Agar contains Maltose as an energy source. Dextrin, a polysaccharide derived from high quality starch, and Glycerol are included as carbon sources. Peptone is provided as a nitrogen source. Bacto Agar is the solidifying agent.

Malt Extract Broth contains Malt Extract Base which provides the carbon, protein, and nutrient sources required for growth of microorganisms. Maltose is added as an energy source. Dextrose is included as a source of fermentable carbohydrate. Yeast Extract provides the vitamins and cofactors required for growth and additional sources of nitrogen and carbon.

The acidic pH of Malt Extract Agar and Broth allow for the optimal growth of molds and yeasts while restricting bacterial growth.

Formula

Malt Extract Agar

Formula per liter

Maltose	12.75 g
Bacto Dextrin	2.75 g
Bacto Glycerol	2.35 g
Bacto Peptone	0.78 g
Bacto Agar	15 g

Final pH 4.7 ± 0.2 at 25°C

Malt Extract Broth

Formula per liter

Malt Extract Base	6 g
Maltose	1.8 g
Bacto Dextrose	6 g
Bacto Yeast Extract	1.2 g

Final pH 4.7 ± 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 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 a product if it fails to meet specifications for identity and performance.

Procedure
Materials Provided
Malt Extract Agar
Malt Extract Broth

Materials Required But Not Provided
Glassware
Autoclave
Incubator

Method of Preparation
1. **Malt Extract Agar**: Suspend 33.6 grams in 1 liter of distilled or deionized water. Heat to boiling to dissolve completely. **Malt Extract Broth**: Dissolve 15 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.

Specimen Collection and Preparation
Refer to appropriate references for specimen collection and preparation.

Test Procedure
See appropriate references for specific procedures.

Results
Refer to appropriate references and procedures for results.

References

1. Abs. Bact., **3**:6, 1919.
2. **Thom, C., and M. B. Church.** 1926. The Aspergilli. Williams and Wilkins Co., Baltimore.
3. **MacFaddin, J.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams and Wilkins, Baltimore.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, MD.

Packaging
Malt Extract Agar	500 g	0112-17
	10 kg	0112-08
Malt Extract Broth	500 g	0113-17
	10 kg	0113-08

Bacto® Mannitol Salt Agar

Intended Use
Bacto Mannitol Salt Agar is used for isolating and differentiating staphylococci.

Summary and Explanation
Chapman[1] formulated Mannitol Salt Agar to isolate staphylococci by inhibiting the growth of most other bacteria with a high salt concentration. He added 7.5% sodium chloride to Phenol Red Mannitol Agar and noted that pathogenic strains of staphylococci (coagulase-positive staphylococci) grew luxuriantly and produced yellow colonies with yellow zones in the surrounding medium. Nonpathogenic staphylococci produced small red colonies with no color change to the surrounding medium.

Because Mannitol Salt Agar is selective, specimens from heavily contaminated sources may be streaked onto this medium without danger of overgrowth[2]. Mannitol Salt Agar is recommended for isolating pathogenic staphylococci from clinical specimens,[2] from cosmetics,[3] and for microbial limit tests.[4]

Principles of the Procedure
Mannitol Salt Agar contains Proteose Peptone No. 3 and Beef Extract as sources of carbon, nitrogen, vitamins and minerals. D-Mannitol is the carbohydrate source. Sodium Chloride, in high concentration, inhibits most bacteria other than staphylococci. Phenol Red is the pH indicator. Bacto Agar is the solidifying agent.

Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products which turn the phenol red pH indicator from red to yellow. Typical pathogenic staphylococci (coagulase-positive staphylococci) ferment mannitol and form yellow colonies with yellow zones around the colonies. Typical non-pathogenic staphylococci do not ferment mannitol and form red colonies.

Formula
Mannitol Salt Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Bacto D-Mannitol	10 g
Sodium Chloride	75 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 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

Mannitol Salt Agar

Materials Required but not Provided

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

Method of Preparation

1. Suspend 111 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

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

Test Procedure

Inoculate specimen onto medium as a primary isolation or inoculate isolated colonies onto medium for differentiation.

Results

Staphylococci will grow on this medium while the growth of most other bacteria will be inhibited. Coagulase-positive staphylococci will produce luxuriant growth of yellow colonies with yellow zones around them. Coagulase negative staphylococci will produce small red colonies with no color change to the medium surrounding them.

References

1. **Chapman, G. H.** The significance of sodium chloride in studies of staphylococci. J. Bacteriol. **50**:201.
2. **Kloos, W. E., and T. L. Bannerman.** 1995. Staphylococcus and Micrococcus. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1995. Microbiology methods for cosmetics, p. 23.01-23.12. In Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.

Packaging

Mannitol Salt Agar	100 g	0306-15
	500 g	0306-17

User Quality Control

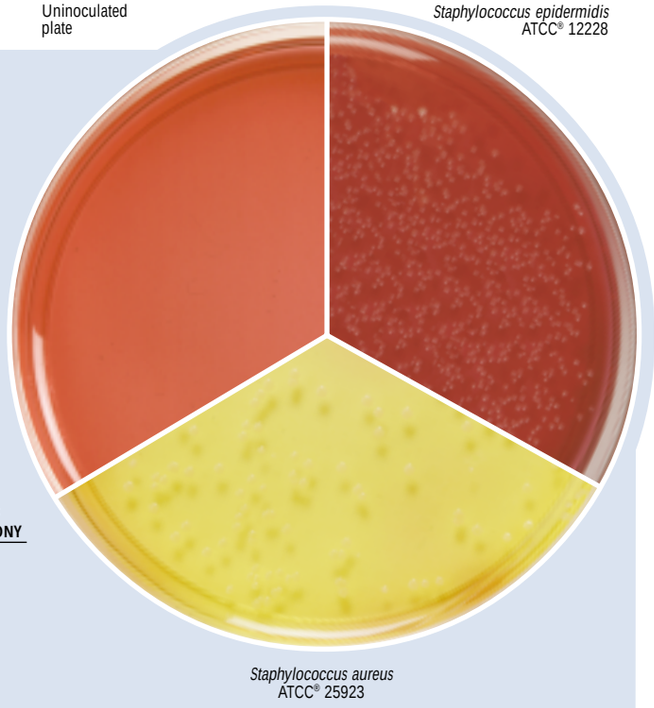
Identity Specifications

Dehydrated Appearance:	Light pink, free-flowing, homogeneous.
Solution:	11.1% solution, soluble in distilled or deionized water on boiling. Solution is red, slightly opalescent.
Prepared Medium:	Pinkish red, slightly opalescent.
Reaction of 11.1% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Mannitol Salt Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 to 48 hours. A yellow zone surrounding a colony indicates mannitol has been fermented.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE OF MEDIUM AND COLONY
Enterobacter aerogenes	13048*	1,000-2,000	marked to complete inhibition	–
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	–
Proteus mirabilis	12453	1,000-2,000	partial inhibition	–
Staphylococcus aureus	25923*	100-1,000	good	yellow colony with yellow zone
Staphylococcus epidermidis	12228*	100-1,000	good	red colony with no zone of color change



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® Marine Agar 2216
Bacto Marine Broth 2216

Intended Use

Bacto Marine Agar 2216 and Bacto Marine Broth 2216 are used for cultivating heterotrophic marine bacteria.

Summary and Explanation

Marine bacteria are present in nutrient sea water by the millions per ml and are essential to the life cycle of all marine flora and fauna. The enumeration and activity of marine bacteria are important to the food industry for the conservation of marine life. Marine Agar 2216 and Marine Broth 2216 are prepared according to the formula of ZoBell[1] The media contain all of the nutrients necessary for the growth of marine bacteria. The media contain minerals that nearly duplicate the major mineral composition of sea water,[2] in addition to Bacto Peptone and Yeast Extract that provide a good source of nutrients.

In the use of Marine Agar 2216, the conventional pour plate and spread plate techniques of enumeration are used. For the pour plate technique, the agar must be cooled to 42°C before inoculation because of the thermo-sensitive nature of most marine bacteria. In the spread plate technique, the agar is poured while hot and allowed to cool and solidify before inoculation. This latter method was reported by Buck and Cleverdon[3] to give higher counts than the pour plate method because of the increased growth of the thermo-sensitive bacteria.

Sizemore and Stevenson[4] used Marine Agar 2216 routinely as the upper nutrient layer of a marine agar-milk agar double-layer plate. This two layer plate was developed for isolating proteolytic marine bacteria. Marine Agar 2216 was also used in studies characterizing a marine bacterium associated with *Crassostrea virginica* (the Eastern Oyster).[5]

Principles of the Procedure

Bacto Peptone and Yeast Extract provide nitrogen, vitamins and minerals.

The high salt content helps to simulate sea water. Numerous minerals are also included to duplicate the major mineral composition of sea water. Bacto Agar is a solidifying agent.

Formula

Marine Agar 2216

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	1 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride	8.8 g
Sodium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide	0.08 g
Strontium Chloride	0.034 g
Boric Acid	0.022 g
Sodium Silicate	0.004 g
Sodium Fluoride	0.0024 g
Ammonium Nitrate	0.0016 g
Disodium Phosphate	0.008 g
Bacto Agar	15 g

Final pH 7.6 ± 0.2 at 25°C

Marine Broth 2216

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	1 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride Dried	5.9 g
Sodium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide	0.08 g
Strontium Chloride	0.034 g
Boric Acid	0.022 g
Sodium Silicate	0.004 g
Sodium Fluoride	0.0024 g
Ammonium Nitrate	0.0016 g
Disodium Phosphate	0.008 g

Final pH 7.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Marine Broth 2216**

   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. TARGET ORGAN(S): Kidneys, Nerves.

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

Marine Agar 2216
Marine Broth 2216

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Glassware
Incubator (20-25°C)
Shaker

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Marine Agar 2216 - 55.1 grams;
   Marine Broth 2216 - 37.4 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Marine Agar 2216: Cool agar to 45-50°C in a waterbath.
5. Dispense.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

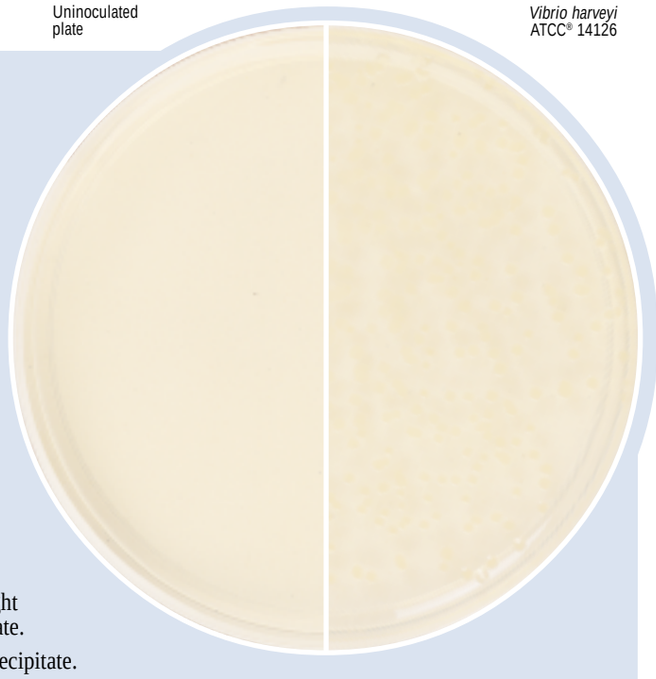
Consult appropriate references for recommended test procedures.[3,4]

Results

Refer to appropriate references and procedures for results.

References

1. **ZoBell, C. E.** 1941. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J. Mar. Res. **4**:42-75.
2. **Lyman, J., and R. H. Fleming.** 1940. Composition of sea water. J. Marine Res. **3**:134.
3. **Buck, J. D., and R. C. Cleverdon.** 1960. The spread plate as a method for the enumeration of marine bacteria. Limnol. Oceanogr. **5**:78.



User Quality Control

Identity Specifications

Marine Agar 2216

Dehydrated Appearance:	Light beige with a few dark particles, free flowing, homogeneous.
Solution:	5.51% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent to opalescent with a precipitate.
Prepared Plates:	Light amber, slightly opalescent to opalescent, may have a slight precipitate.
Reaction of 5.51% Solution at 25°C:	pH 7.6 ± 0.2

Marine Broth 2216

Dehydrated Appearance:	Light beige with a few dark particles, free flowing.
Solution:	3.74% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent with a precipitate.
Prepared Flasks:	Light amber, slightly opalescent with a precipitate.
Reaction of 3.74% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Prepare Marine Agar 2216 per label directions. Inoculate and incubate at 20-25°C for 40-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Vibrio fischeri	7744	100-1,000	good
Vibrio harveyi	14126	100-1,000	good

Prepare Marine Broth 2216 per label directions. Dispense 50 ml amounts in 250 ml Erlenmeyer flasks. Inoculate and incubate at 20-25°C on a shaker for 40-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Vibrio fischeri	7744	100-1,000	good
Vibrio harveyi	14126	100-1,000	good

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

4. **Sizemore, R. K., and L. H. Stevenson.** 1970. Method for the isolation of proteolytic marine bacteria. Appl. Microbiol. **20**:991-992.
5. **Weiner, R. M., A. M. Segall, and R. R. Colwell.** 1985. Characterization of a marine bacterium associated with *Crassostrea virginica* (the Eastern Oyster). Appl. Environ. Microbiol. **49**:83-90.

Packaging

Marine Agar 2216	500 g	0979-17
Marine Broth 2216	500 g	0791-17

Bacto® Maximum Recovery Diluent

Intended Use

Bacto Maximum Recovery Diluent is an isotonic diluent containing a low level of peptone used for maintaining the viability of organisms during dilution procedures.

Summary and Explanation

Standard methods for the microbiological examination of foodstuffs require sample dilution to be carried out accurately to estimate the number of microorganisms. Diluents consisting of sterile saline, phosphate buffer solutions and distilled water have all been shown to have a lethal action on a wide range of organisms.[1,2]

The presence of low levels of peptone in the diluent at a pH of 7.0 ± 0.2 affords protection for bacteria for at least one hour during the dilution stage.[3,4] The presence of peptone also allows accurate quantitative procedures to be performed with minimal reductions in viable count in the diluent.

Principles of Procedure

Low levels of peptone help protect organisms in the diluent. Sodium Chloride maintains proper osmotic pressure.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Cream, free-flowing, homogeneous.
Solution:	0.95% solution, soluble in distilled or deionized water, colorless, clear.
Prepared Medium:	Colorless, clear.
Reaction of 0.95% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare the medium per label directions. Inoculate tubes with the test organism. At time zero and after 30 minutes, subculture 1.0 ml aliquots into Tryptic Soy Agar, using the pour plate technique. Incubate plates at 35°C for 18-24 hours.

ORGANISM	ATCC®	RECOVERY AFTER 30 MINUTES
Escherichia coli	25922*	no significant reduction
Staphylococcus aureus	25923*	no significant reduction

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

Formula

Formula per liter

Bacto Peptone	1 g
Sodium Chloride	8.5 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Maximum Recovery Diluent

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave

Method of Preparation

1. Dissolve 9.5 grams in 1 liter distilled or deionized water.
2. Dispense into final containers and cap loosely.
3. Autoclave at 121°C for 15 minutes.

Test Procedure

Consult appropriate references for dilution procedures when testing foods.[1,2,3,4]

Results

Refer to appropriate references and procedures for results.

References

1. **DeMello, G. C., I. S. Danielson, and J. S. Kiser.** 1951. The Toxic effect of buffered saline on the viability of *Brucella abortus*. J. Lab. Clin. Med. **37**:579-583.

2. **Gunter, S. E.** 1954. Factors determining the viability of selected microorganisms in inorganic media. J. Bacteriol. **67**:628-634.
3. **Straka, R. P., and J. L. Stokes.** 1957. Rapid destruction of bacteria in commonly used diluents and its eliminations. Appl. Microbiol. **5**:21-25.
4. **Patterson, J. T., and J. A. Cassells.** 1963. An examination of the value of adding peptone to diluents used in the bacteriological testing of bacon curing brines. J. Appl. Bacteriol. **26**:493-497.

Packaging

Maximum Recovery Diluent	500 g	1897-17
	5 kg	1897-03

Bacto® McBride Listeria Agar

Intended Use

Bacto McBride Listeria Agar is used for isolating *Listeria monocytogenes* with or without the addition of blood.

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. *Listeria* species are ubiquitous in nature, being present in a wide range of unprocessed foods, 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 of these parameters.[7] *Listeria* species 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.

McBride Listeria Agar is prepared according to the formulation of McBride and Girard,[9] who originally described the medium and its use in the selective isolation of *Listeria* from experimentally mixed cultures. The medium has been recommended for isolating *Listeria* from clinical specimens,[10] raw milk[11,12] and food samples.[13]

When the medium is used as a blood agar, a narrow zone of beta-hemolysis may be evident around and under *Listeria* colonies.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, homogeneous with soft lumps.
Solution:	4.6% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium without Blood:	Light to medium amber, slightly opalescent.
Prepared Medium with Blood:	Cherry red, opaque.
Reaction of 4.6% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition
Listeria monocytogenes	19114	100-1,000	good

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

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

Principles of the Procedure

Tryptose and Beef Extract provide nitrogen, vitamins and minerals. Sodium Chloride maintains the osmotic balance of the medium. Partial selectivity is provided by Lithium Chloride, Glycine and Phenylethanol, which aid in suppressing both gram-positive and gram-negative bacteria other than *Listeria*. If increased inhibition of fungi is also needed, cycloheximide can be added after autoclaving. Bacto Agar is a solidifying agent.

Formula

McBride Listeria Agar

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Sodium Chloride	5 g
Glycine	10 g
Lithium Chloride	0.5 g
Phenylethanol	2.5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

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

Storage

Store the dehydrated medium at 2-8°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

McBride Listeria Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 46 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 medium to 45-50°C in a waterbath.
5. To enhance selectivity and/or differentiation, aseptically add cycloheximide (0.2 grams/liter) and/or sterile defibrinated blood to the medium. Mix well.
6. Dispense into sterile 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.[10,12,13]
2. Process each specimen, using procedures appropriate for that specimen or sample.[10,12,13]

Test Procedure

When testing clinical specimens for *Listeria*, inoculate directly onto primary plating media and McBride Listeria Agar.[10]

When isolating *Listeria* from raw milk and food samples, refer to appropriate references.[12,13]

Results

Observe colonies under oblique transmitted light. *Listeria* colonies should 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.
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8. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
9. **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.
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11. **Hayes, P. S., J. C. Feeley, L. M. Graves, G. W. Ajello, and D. W. Fleming**. 1986. Isolation of *Listeria monocytogenes* from raw milk. Appl. Environ. Microbiol. **51**:438-440.
12. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products. *In* R. T. Marshall (ed), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

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

Packaging

McBride Listeria Agar	500 g	0922-17

McClung Toabe Agar

Bacto® McClung Toabe Agar Base · Bacto Egg Yolk Enrichment 50%

Intended Use

Bacto McClung Toabe Agar Base is used with Bacto Egg Yolk Enrichment 50% for isolating and detecting *Clostridium perfringens* in foods based on the lecithinase reaction.

Summary and Explanation

McClung and Toabe[1] formulated a medium for isolating *C. perfringens* from foods. With the addition of 50% egg yolk emulsion, *C. perfringens* and a few other *Clostridium* species show the lecithinase reaction.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients, but occurrences of food borne illness are usually associated with cooked meat or poultry products.[2] Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.[3] Enumerating the microorganism in food samples plays a role in epidemiological investigation of outbreaks of food borne illness.[2]

User Quality Control

Identity Specifications

McClung Toabe Agar Base

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	7.5% solution, soluble in distilled or deionized water on boiling. Solution is light amber, opalescent, with a precipitate.
Prepared Medium:	Light yellow, smooth, opaque.
Reaction of 7.5% Solution at 25°C:	pH 7.6 ± 0.2

Egg Yolk Enrichment 50%

Appearance:	Canary yellow, opaque liquid with a resuspendable precipitate.

Cultural Response

McClung Toabe Agar Base with Egg Yolk Enrichment 50%

Prepare McClung Toabe Agar Base with Egg Yolk Enrichment 50% per label directions. Inoculate and incubate the plates at 35 ± 2°C anaerobically for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	LECITHINASE REACTION
Clostridium perfringens	12919	100-1,000	good	opaque halo
Clostridium perfringens	12924	100-1,000	good	opaque halo
Staphylococcus aureus	25923*	100-1,000	good	opaque halo
Staphylococcus epidermidis	14990	100-1,000	good	none

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.

Principles of the Procedure

McClung Toabe Agar Base contains Proteose Peptone as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate source. Sodium Chloride maintains the osmotic balance of the medium. Magnesium Sulfate provides divalent cations and sulfate. Sodium Phosphate Dibasic and Potassium Phosphate Monobasic maintain pH balance and provide a source of phosphates. Bacto Agar is the solidifying agent. Egg Yolk Enrichment 50% provides egg yolk lecithin. Lecithinase-producing clostridia, such as *C. perfringens*, hydrolyze the lecithin and produce opaque halos.

Formula

McClung Toabe Agar Base

Formula Per Liter

Bacto Proteose Peptone	40 g
Bacto Dextrose	2 g
Sodium Phosphate Dibasic	5 g
Potassium Phosphate Monobasic	1 g
Sodium Chloride	2 g
Magnesium Sulfate	0.1 g
Bacto Agar	25 g

Final pH 7.6 ± 0.2 at 25°C

Egg Yolk Enrichment 50%

Sterile concentrated egg yolk emulsion

Precautions

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

Storage

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

Store the Egg Yolk Enrichment 50% 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

McClung Toabe Agar Base
Egg Yolk Enrichment 50%

Materials Required but not Provided

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

Method of Preparation

McClung Toabe Agar Base

1. Suspend 75 grams of McClung Toabe Agar Base in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 90 ml amounts into flasks.
4. Autoclave at 121°C for 20 minutes. Cool to 50°C.
5. Aseptically add 10 ml Egg Yolk Enrichment 50% to each flask of prepared agar base.
6. Mix thoroughly.
7. Pour into sterile Petri dishes in approximately 15 ml amounts.

Egg Yolk Enrichment 50%

1. Ready for use.
2. Shake gently to resuspend precipitate.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **McClung, L. S., and R. Toabe**. 1947. The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. J. Bact. **53**:139.
2. **Labbe, R. G., and S. M. Harmon.** 1992. *Clostridium perfringens*, p. 623-635. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Rhodehamel, E. J., and S. M. Harmon**. 1995. *Clostridium perfringens*, p. 16.01- 16.06. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

McClung Toabe Agar Base	500 g	0941-17
Egg Yolk Enrichment 50%	12x10 ml	3347-61*
	6x100 ml	3347-73*

*Store at 2-8°C

Methyl Red and Voges-Proskauer Tests

Bacto® MR-VP Medium · SpotTest™ Voges-Proskauer Reagent A SpotTest Voges-Proskauer Reagent B

Intended Use

Bacto MR-VP Medium is used for differentiating coliform organisms based on the methyl red and Voges-Proskauer tests.

SpotTest Voges-Proskauer Reagents A and B are used for determining the VP reaction of bacteria.

Also Known As

MR-VP Medium is also known as Methyl Red-Voges Proskauer Medium.

Summary and Explanation

In 1915, Clark and Lubs[1] demonstrated that the colon-aerogenes family of bacteria could be divided into two groups based on their action in a peptone and dextrose medium. When tested with the pH indicator methyl red, the "coli" group produced high acidity while the "aerogenes" group produced a less acid reaction. The test to detect high-acid end products is known as the Methyl Red (MR) test. The test to detect less-acid end products is based on the procedure described by Voges and Proskauer in 1898.[2] A color reaction occurs when certain cultures, incubated in a medium containing peptone and dextrose, are treated with potassium hydroxide and exposed to air. This reaction detects the formation of acetylmethylcarbinol and is known as the Voges-Proskauer (VP) Test.

The MR and VP tests appear in the identification scheme for the *Enterobacteriaceae*,[3] which are important isolates in clinical microbiology,[3] as well as in the microbiology of foods and dairy products.[4,5]

The MR and VP tests are used to complete and confirm the identification of *Escherichia coli*. [4,5,6]

Principles of the Procedure

MR-VP Medium contains Buffered Peptone as a carbon and nitrogen source for general growth requirements. Dextrose is a fermentable carbohydrate.

Members of the *Enterobacteriaceae* convert glucose to pyruvate by the Embden-Meyerhof pathway. Some bacteria metabolize pyruvate by the mixed acid pathway and produce acidic end products (pH < 4.4), such as lactic, acetic and formic acids. Other bacteria metabolize pyruvate by the butylene glycol pathway and produce neutral end products (pH > 6.0), one of which is acetoin (acetylmethylcarbinol). In the MR test, the pH indicator methyl red detects acidic end products.[7] In the VP test, acetoin is oxidized in the presence of oxygen and potassium hydroxide (KOH) to diacetyl, which produces a red color.[8]

User Quality Control

Identity Specifications

MR-VP Medium

Dehydrated Appearance:	Very light to light beige, free-flowing, homogeneous.
Solution:	1.7% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 1.7% Solution at 25°C:	pH 6.9 ± 0.2

SpotTest Voges-Proskauer Reagent A

Appearance:	Yellow to dark amber, clear solution inside a glass ampule contained in a plastic dispenser.

SpotTest Voges-Proskauer Reagent B

Appearance:	Colorless, clear solution inside a glass ampule contained in a plastic dispenser.

Cultural Response

MR-VP Medium, SpotTest Voges-Proskauer Reagent A or SpotTest Voges-Proskauer Reagent B

Prepare MR-VP Medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours or up to 5 days. Determine the methyl red and Voges-Proskauer test reactions.

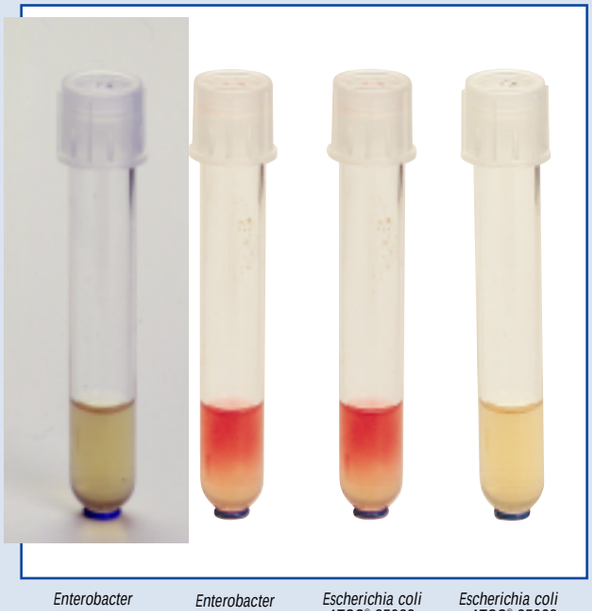
ORGANISM	ATCC®	INOCULUM	GROWTH	APPEARANCE MR TEST	APPEARANCE VP TEST
Enterobacter aerogenes	13048*	undiluted	good	–/yellow	+/red
Escherichia coli	25922*	undiluted	good	+/red	–/no change

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 | *Enterobacter aerogenes* ATCC® 13048 | *Escherichia coli* ATCC® 25922

MR-VP Medium



Enterobacter aerogenes ATCC® 13048 MR Test | *Enterobacter aerogenes* ATCC® 13048 VP Test | *Escherichia coli* ATCC® 25922 MR Test | *Escherichia coli* ATCC® 25922 VP Test

The addition of -naphthol (SpotTest Voges-Proskauer Reagent A) before KOH (SpotTest Voges-Proskauer Reagent B) enhances the sensitivity of the test.[8]

Formula

MR-VP Medium

Formula Per Liter

Buffered Peptone	7 g
Dipotassium Phosphate	5 g
Bacto Dextrose	5 g

Final pH 6.9 ± 0.2 at 25°C

SpotTest Voges-Proskauer Reagent A

Formula Per Liter

α-Naphthol	50 g
Ethyl Alcohol (absolute)	1,000 ml

SpotTest Voges-Proskauer Reagent B

Formula Per Liter

Potassium Hydroxide	400 g
Distilled Water	1,000 ml

Precautions

1. For Laboratory Use.
2. **SpotTest Voges-Proskauer Reagent A**

   HIGHLY FLAMMABLE. IRRITANT. HIGHLY FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist. Keep away from sources of ignition. No smoking. Keep container tightly closed. Target Organs: Liver, Blood.

   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.

   SpotTest Voges-Proskauer Reagent B

   CORROSIVE. CAUSES SEVERE BURNS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

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

Storage

Store the dehydrated MR-VP Medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store SpotTest Voges-Proskauer Reagents A and B at 15-30°C. Protect from light.

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

MR-VP Medium
SpotTest Voges-Proskauer Reagent A
SpotTest Voges-Proskauer Reagent B

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35 ± 2°C)
Test tubes with caps
Test tubes, 13 x 100 mm
Methyl red indicator[7]

Method of Preparation

MR-VP Medium

1. Dissolve 17 grams in 1 liter distilled or deionized water.
2. Distribute into test tubes. Autoclave at 121°C for 15 minutes.

Methyl Red Indicator

1. Dissolve 0.1 gram of methyl red in 300 ml of 95% ethyl alcohol.
2. Add sufficient distilled or deionized water to make 500 ml.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate MR-VP Medium with growth from a single colony.
2. Incubate at 35 ± 2°C for 48 hours.
3. Test as follows.

Methyl Red Test

1. Transfer 2.5 ml of the MR-VP Medium culture to a tube (13 x 100 mm).
2. Add 5 drops of methyl red indicator and observe for a color change.

VP Test

1. Transfer 2.5 ml of the MR-VP Medium culture to a tube (13 x 100 mm).
2. Add 0.3 ml (6 drops) of SpotTest Voges-Proskauer Reagent A (5% α-naphthol).
3. Add 0.1 ml (2 drops) of SpotTest Voges-Proskauer Reagent B (40% KOH).
4. Gently agitate the tube and let stand for 10 to 15 minutes.
5. Observe for a color change.

Other Methods

SpotTest Voges-Proskauer Reagent A and Reagent B are suitable for use in other modifications of the Voges-Proskauer test requiring the use of these reagents.

Alternate VP Tube Method

1. Inoculate MR-VP Medium with the test organism and incubate at 35°C for 24-48 hours.
2. Aseptically transfer 1 ml of the incubated MR-VP Medium culture to a clean test tube.
3. Add 15 drops of Voges-Proskauer Reagent A followed by 5 drops of Voges-Proskauer Reagent B.
4. Shake gently to aerate.
5. Examine for the appearance of a red color within 20 minutes.
6. If the 24-hour test is negative, repeat the test with a 48-hour culture of the test organism. If equivocal results are obtained, repeat the test with cultures incubated for 5 days at 25-30°C.

Rapid Micro Method

1. Inoculate 0.2 ml of MR-VP Medium with the test organism.
2. Incubate for 4 hours at 35°C.
3. Add 0.1 ml of 0.3% creatine solution.
4. Add 5 drops of Voges-Proskauer Reagent A followed by 2 drops of Voges-Proskauer Reagent B.
5. Shake gently to aerate.
6. Examine for the appearance of a red color within 20 minutes.

Results

Methyl Red (MR) Test

Positive:	Bright red color.
Negative:	Yellow-orange color.

Note: If the test is negative, continue to incubate the broth without added reagent; repeat the test after an additional 18 to 24 hours incubation.

Voges-Proskauer (VP) Test

Positive:	Red color.
Negative:	No red color.

Limitations of the Procedure

1. Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within the *Enterobacteriaceae*.
2. A precipitate may form in the potassium hydroxide reagent solution. This precipitate has not been shown to reduce the effectiveness of the reagent.
3. Most members of the family *Enterobacteriaceae* give either a positive MR test or a positive VP test. However, certain organisms such as *Hafnia alvei* and *Proteus mirabilis* may give a positive result for both tests.
4. Incubation time for the Methyl Red test cannot be shortened by increasing the glucose concentration in the medium or by heavily inoculating the broth.[7]
5. Incubate MR-negative tests for more than 48 hours and test again. (See **Results** section.)
6. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.[8]
7. VP reagents must be added in the order and the amounts specified or a weak-positive or false-negative reaction may occur. A weak-positive reaction may be masked by a copper-like color which may form due to the reaction of KOH and α-naphthol.[8]
8. Read the VP test within 1 hour of adding the reagents. The KOH and α-naphthol may react to form a copper-like color, causing a potential false-positive interpretation.[8]
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials,[9] the use of media low in these substances (such as MR-VP Medium) is recommended for this test.

References

1. **Clark, W. M., and H. A. Lubs.** 1915. The differentiation of bacteria of the colon- aerogenes family by the use of indicators. J. Infect. Dis. **17**:160-173.
2. **Voges, O., and B. Proskauer.** 1898. Z. Hyg. **28**:20-22.
3. **Farmer, J. J., III**. 1995. Enterobacteriaceae: Introduction and identification, p. 438-449. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
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 **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, sup. 1, 1.19.48. American Society for Microbiology, Washington, D.C.
8. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, sup. 1, 1.19.58. American Society for Microbiology, Washington, D.C.
9. **Barritt, M. M.** 1936. The intensification of the Voges-Proskauer reaction by the addition of alpha-naphthol. J. Pathol. **42**:441-454.

Packaging

MR-VP Medium	100 g	0016-15
	500 g	0016-17
	2 kg	0016-07
SpotTest Voges-Proskauer Reagent A	50 x 0.75 ml	3558-26
SpotTest Voges-Proskauer Reagent B	50 x 0.75 ml	3559-26

Bacto® Micro Assay Culture Agar
Bacto Micro Inoculum Broth

Intended Use

Bacto Micro Assay Culture Agar is used for cultivating lactobacilli and other organisms used in microbiological assays.

Bacto Micro Inoculum Broth is used for preparing the inoculum of lactobacilli and other microorganisms used in microbiological assays of vitamins and amino acids.

Summary and Explanation

Three types of media are used in the microbiological assay of vitamins:

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

Micro Assay Culture Agar is used for maintaining stock cultures of lactobacilli and other test microorganisms. This medium is also used for general cultivation of lactobacilli.

Micro Inoculum Broth is used for cultivating lactobacilli and preparing the inoculum for microbiological assays.

User Quality Control

Identity Specifications

Micro Assay Culture Agar

Dehydrated Appearance:	Light tan to tan, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 6.7 ± 0.2

Micro Inoculum Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.7% solution, soluble in distilled or deionized water. Light to medium amber in color, clear to very slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, clear to very slightly opalescent.
Reaction of 3.7% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Micro Assay Culture Agar and Micro Inoculum Broth per label directions. Inoculate tubes with test organisms. Incubate Micro Assay Culture Agar at 35 ± 2°C for 18-48 hours, incubate Micro Inoculum Broth at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus hirae	804	100-1,000	good
Lactobacillus casei subsp. *rhamnosus*	7469	100-1,000	good
Lactobacillus delbrueckii subsp. *lactis*	7830	100-1,000	good
Lactobacillus plantarum	8014	100-1,000	good

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

Principles of the Procedure

Proteose Peptone No. 3 provides nitrogen and amino acids in both Micro Assay Culture Agar and Micro Inoculum Broth. Yeast Extract is a vitamin source. Dextrose is a carbon source. Monopotassium phosphate is a buffering agent. Sorbitan monooleate complex (Micro Inoculum Broth) and Polysorbate 80 (Micro Assay Culture Agar) act as emulsifiers. Bacto Agar is a solidifying agent (Micro Assay Culture Agar).

Formula

Micro Assay Culture Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	20 g
Bacto Dextrose	10 g
Monopotassium Phosphate	2 g
Polysorbate 80	0.1 g
Bacto Agar	10 g

Final pH 6.7 ± 0.2 at 25°C

Micro Inoculum Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	20 g
Bacto Dextrose	10 g
Monopotassium Phosphate	2 g
Sorbitan Monooleate Complex	0.1 g

Final pH 6.7 ± 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 and glassware used in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used.

Storage

Store the dehydrated media below 30°C. The 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.

Procedure

Materials Provided

Micro Assay Culture Agar
Micro Inoculum Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Inoculating needle
0.9% NaCl

Method of Preparation

Micro Assay Culture Agar

1. Suspend 47 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve.
3. Dispense 10 ml amounts into 16-20 mm diameter tubes.
4. Autoclave at 121°C for 15 minutes.
5. Agitate tubes prior to solidification to disperse the flocculent precipitate.

Micro Inoculum Broth

1. Dissolve 37 grams in 1 liter distilled or deionized water.
2. Dispense 10 ml amounts into tubes of 16-20 mm diameter.
3. Autoclave at 121°C for 15 minutes.

Stock Cultures

1. Prepare stock cultures in triplicate on Micro Assay Culture Agar, inoculating tubes using a straight-wire inoculating needle.
2. Incubate tubes at 30-37°C for 18-24 hours.
3. Store at 2-8°C.
4. Transfer cultures at weekly or twice-monthly intervals.

Assay Inoculum

1. Subculture from a 16-24 hour stock culture of lactobacilli in Micro Assay Culture Agar into a 10 ml tube of Micro Inoculum Broth.
2. Incubate at 35-37°C for 16-24 hours or as specified in the assay procedure.
3. Centrifuge the culture and decant the supernatant.
4. Resuspend cells in 10 ml of sterile 0.9% NaCl solution or sterile single strength basal assay medium.
5. Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise indicated.
6. Dilute the washed suspension 1:100 with sterile 0.9% single strength basal assay medium or as indicated. Where applicable, adjust inoculum concentration according to limits specified in AOAC[1] or US Pharmacopeia.[2]

Specimen Collection and Preparation

Prepare samples for assay according to references given in the specific assay procedure. Dilute assay samples to approximately the same concentration as the standard solution.

Test Procedure

For a complete discussion of vitamin assay methodology, refer to appropriate procedures.[1,2]

Results

For test results on vitamin assay procedures, refer to appropriate procedures.[1,2]

Limitations of the Procedure

1. Test organisms used in assay procedures must be cultured and maintained on media recommended for this purpose.
2. Follow assay directions exactly. The age, preparation and size of inoculum are extremely important factors in obtaining a satisfactory assay result.
3. Although other media and methods may be used successfully for maintaining cultures and preparing inocula, uniformly good results will be obtained if the methods described are followed exactly.
4. Aseptic technique should be used throughout the microbiological assay procedure.
5. The use of altered or deficient media may create mutants having different nutritional requirements. Such organisms will not produce a satisfactory test response.

References

1. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
2. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.

Packaging

Micro Assay Culture Agar	100 g	0319-15
	500 g	0319-17
Micro Inoculum Broth	500 g	0320-17

Bacto® Microbial Content Test Agar

Intended Use

Bacto Microbial Content Test Agar is recommended for the detection of microorganisms on surfaces sanitized with quaternary ammonium compounds.

Also Known as

"Tryptic Soy Agar with Lecithin and Polysorbate 80" (TSALT) and "Casein Soy Peptone Agar with Polysorbate 80 and Lecithin" are common terms for Microbial Content Test Agar. Tween 80® is also known as Polysorbate 80.

Summary and Explanation

Microbial Content Test Agar is a modification of Tryptic Soy Agar with Lecithin and Tween 80. The formulation is recommended for determining the sanitation efficiency of containers, equipment and work areas (environmental monitoring). The Lecithin and Tween in the formula inactivate some preservatives that may inhibit bacterial

growth, reducing "preservative carryover."[1] The formulation is recommended for the Aerobic Plate Count (Microbial Limit Test) for water miscible cosmetic products containing preservatives.[1]

Principles of the Procedure

Microbial Content Test Agar contains Tryptone and Soytone which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Lecithin and Polysorbate 80 are added to neutralize surface disinfectants.[2,3,4] Lecithin is added to neutralize quaternary ammonium compounds. Polysorbate 80 is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol.[5] Sodium Chloride provides osmotic equilibrium. Bacto Agar has been incorporated into this medium as a solidifying agent.

Formula

Microbial Content Test Agar

Formula Per Liter

Bacto Tryptone	15 g
Bacto Soytone	5 g
Sodium Chloride	5 g
Lecithin	0.7 g
Polysorbate 80	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous, may appear moist.
Solution:	4.57% solution soluble in distilled or deionized water on boiling with frequent gentle swirling. At approximately 50°C, solution is medium amber in color, slightly opalescent, with a resuspendable precipitate. At higher temperatures, it is more opalescent.
Prepared Medium:	Medium amber, slightly opalescent, may have a precipitate.
Reaction of 4.57% Solution at 25°C	pH 7.3 ± 0.2

Cultural Response

Prepare medium per label directions. Test Microbial Content Test Agar in parallel with Plate Count Agar. Inoculate liquid media with test organisms and pour plates. After the plates dry, apply disks impregnated with varying dilutions of a quaternary ammonium compound to the medium surface. Incubate plates at 35 ± 2°C for 40-48 hours and inspect for zones of inhibition.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	11229	100-1,000	smaller zone of inhibition of growth compared to Plate Count Agar
Staphylococcus aureus	6538P	100-1,000	smaller zone of inhibition of growth compared to Plate Count Agar

Interpretation: The smaller zones of inhibition indicate neutralization of quaternary ammonium compounds by Microbial Content Test Agar.

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.

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

Microbial Content Test Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Sterile Petri dishes

Method of Preparation

1. Suspend 45.7 g in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely with frequent careful agitation to dissolve, 1-2 minutes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Consult appropriate references.[1,4]

Test Procedures

Microbial Content Test Agar is used in a variety of procedures. Consult appropriate references for further information.[1,4]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on the medium.
2. The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

References

1. **Orth, D. S.** 1993. Handbook of Cosmetic Microbiology. Marcel Dekker, Inc., New York, NY.
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 **Erlandson, A. L., Jr., and C. A. Lawrence**. 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science **118:**274-276.
4. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. App. Environ. Microbiol. **32:**80-84.

5. **Favero (chm.)**. 1967. Microbiological sampling of surfaces - a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Packaging

Microbial Content Test Agar	500 g	0553-17
	2 kg	0553-07

Bacto® Middlebrook 7H9 Broth · Bacto Middlebrook 7H10 Agar Bacto Mycobacteria 7H11 Agar · Bacto Middlebrook ADC Enrichment · Bacto Middlebrook OADC Enrichment · Bacto Middlebrook OADC Enrichment w/WR 1339 · Bacto Glycerol

Intended Use

Bacto Middlebrook 7H9 Broth is used with Bacto Middlebrook ADC Enrichment and Bacto Glycerol or Bacto Tween® 80 for cultivating pure cultures of mycobacteria and preparing the tubercle emulsion for susceptibility testing.

Bacto Middlebrook 7H10 Agar is used with Bacto Middlebrook OADC Enrichment and Bacto Glycerol for isolating, cultivating and susceptibility testing of mycobacteria. The complete, prepared medium is also available.

Bacto Middlebrook 7H10 Agar is also used with Bacto Middlebrook OADC Enrichment w/WR 1339 to demonstrate cording in differentiating *Mycobacterium tuberculosis* from atypical mycobacteria.

Bacto Mycobacteria 7H11 Agar is used with Bacto Middlebrook OADC Enrichment and Bacto Glycerol for isolating, cultivating and susceptibility testing of fastidious strains of mycobacteria. The complete, prepared medium is also available.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1-3]

Because mycobacteria grow more rapidly in a broth medium, primary culture of all specimens in a broth medium such as Middlebrook 7H9 Broth is recommended.[6]

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

Egg-base media:

1. Support a wide variety of groups and species of mycobacteria;
2. Provide mycobacterial growth that can be used for niacin testing;
3. Have long shelf lives when refrigerated.[3]

User Quality Control

Identity Specifications

Middlebrook 7H9 Broth

Dehydrated Appearance:	Light beige, free-flowing, homogenous.
Solution:	0.47% solution, soluble in distilled or deionized water. Solution is very light amber, clear.
Prepared Medium:	Colorless to very light amber, clear.
Reaction of 0.47% Solution at 25°C:	6.6 ± 0.2

Middlebrook 7H10 Agar

Dehydrated Appearance:	Light beige with green tint, free-flowing, homogenous.
Solution:	1.9% solution, soluble in distilled or deionized water on boiling. Solution is light amber with a slight green tint, slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent without precipitate.
Reaction of 1.9% Solution at 25°C:	pH 6.6 ± 0.2

Mycobacteria 7H11 Agar

Dehydrated Appearance:	Light beige with a green tint, free-flowing, homogenous.
Solution:	0.21% solution, soluble in distilled or deionized water on boiling. Solution is light yellowish green, slightly opalescent.
Prepared Medium:	Light amber with a greenish tint, slightly opalescent, no precipitate.
Reaction of 0.21% Solution:	pH 6.6 ± 0.2

Middlebrook OADC Enrichment

Appearance:	Light amber, clear solution.

Middlebrook ADC Enrichment

Appearance:	Very light to light amber, clear solution.

Middlebrook OADC Enrichment w/WR 1339

Appearance:	Light amber, clear solution.

Glycerol

Identity Test:	ID positive; IR Spectrum comparable to reference Glycerin.
Appearance:	Colorless, clear, syrupy liquid.

continued on following page

User Quality Control cont.

Cultural Response

Middlebrook 7H9 Broth with Middlebrook ADC Enrichment

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium fortuitum	6841	100-300	good
Mycobacterium intracellulare	13950	100-300	good
Mycobacterium kansasii	12478	100-300	good
Mycobacterium tuberculosis	25177	100-300	good
Mycobacterium scrofulaceum	19981	100-300	good

Middlebrook 7H10 Agar with Middlebrook OADC Enrichment
Mycobacteria 7H11 Agar with Middlebrook OADC Enrichment

Prepare medium per label directions or use prepared tubes. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	1,000-2,000	markedly inhibited
Mycobacterium fortuitum	6841	100-300	good
Mycobacterium intracellulare	13950	100-300	good
Mycobacterium kansasii	12478	100-300	good
Mycobacterium scrofulaceum	25177	100-300	good
Mycobacterium tuberculosis	19981	100-300	good

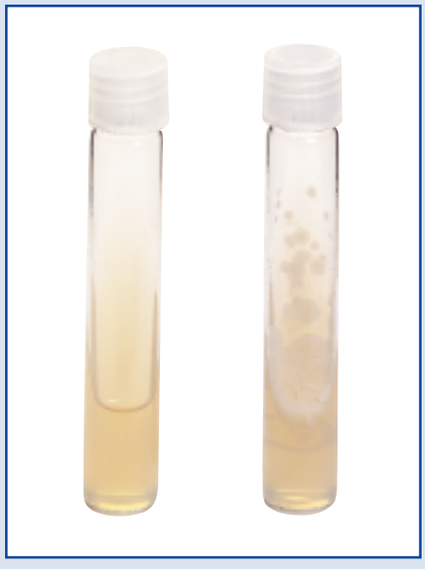
Middlebrook 7H10 Agar with Middlebrook OADC Enrichment w/WR 1339

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for up to three weeks.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium tuberculosis	27294	100-1,000	good

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

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



Uninoculated tube — *Mycobacterium fortuitum* ATCC® 6841

Agar-base media:

1. Tend not to liquefy in the presence of contaminating proteolytic organisms;[3]
2. Are recommended for specimens from nonsterile sites[7] because colonies of mycobacteria can be viewed in a clear medium after 10-12 days incubation using a stereo microscope even if contaminating organisms are present;
3. Retain exact concentrations of added drugs because the medium is solidified with agar rather than by inspissation of the egg. Also, there is less drug inactivation when egg ingredients are absent.

Middlebrook 7H10 Agar is prepared according to Middlebrook, Cohn, Dye, Russell and Levy.[4] This medium contains a low concentration of malachite green, which may be preferable for primary isolation.

Mycobacteria 7H11 Agar is a modification of Middlebrook 7H10 Agar Special as recommended by Cohn, Waggoner and McClately.[5] Cohn et al. demonstrated that the addition of an enzymatic digest of casein stimulates growth of the more fastidious strains of *Mycobacterium tuberculosis* and provides improved susceptibility testing.

Principles of the Procedure

L-Glutamic Acid, Ammonium Sulfate, Biotin, Sodium Citrate and Pyridoxine supply growth factors. Magnesium Sulfate, Ferric Ammonium Sulfate, Zinc Sulfate and Copper Sulfate are sources of trace ions. Disodium Phosphate and Monopotassium Phosphate help to maintain the pH of the medium. Malachite Green inhibits contaminating organisms. Pancreatic Digest of Casein, a good source of nitrogen and carbon, increases the recovery of isoniazid-resistant mycobacteria on Mycobacteria 7H11 Agar. Glycerol enhances the growth of *Mycobacterium avium* as well as other *Mycobacterium* species.[7] Bacto Agar is a solidifying agent.

Middlebrook OADC and ADC Enrichments contain Dextrose and Oleic Acid as carbon sources. Albumin Fraction V, Bovine and Catalase (Beef) are growth factors. WR 1339, Triton® encourages the demonstration of cording in *Mycobacterium tuberculosis*.[8]

Formula

Middlebrook 7H9 Broth

Formula Per Liter

Ammonium Sulfate	0.5 g
L-Glutamic Acid	0.5 g
Sodium Citrate	0.1 g
Pyridoxine	0.001 g
Biotin	0.0005 g
Disodium Phosphate	2.5 g
Monopotassium Phosphate	1 g
Ferric Ammonium Citrate	0.04 g
Magnesium Sulfate	0.05 g
Calcium Chloride	0.0005 g
Zinc Sulfate	0.001 g
Copper Sulfate	0.001 g

Final pH 6.6 ± 0.2 at 25%C

Middlebrook 7H10 Agar

Formula Per Liter

Ammonium Sulfate	0.5 g
Monopotassium Phosphate	1.5 g
Disodium Phosphate	1.5 g
Sodium Citrate	0.4 g

Magnesium Sulfate	0.025 g
Calcium Chloride	0.0005 g
Zinc Sulfate	0.001 g
Copper Sulfate	0.001 g
L-Glutamic Acid (Sodium Salt)	0.5 g
Ferric Ammonium Citrate	0.04 g
Pyridoxine Hydrochloride	0.001 g
Biotin	0.0005 g
Malachite Green	0.00025 g
Bacto Agar	15 g

Final pH 6.6 ± 0.2 at 25%C

Mycobacteria 7H11 Agar

Formula Per Liter

Pancreatic Digest of Casein	1 g
L-Glutamic Acid	0.5 g
Sodium Citrate	0.4 g
Pyridoxine	0.001 g
Biotin	0.0005 g
Ferric Ammonium Citrate	0.04 g
Ammonium Sulfate	0.5 g
Disodium Phosphate	1.5 g
Monopotassium Phosphate	1.5 g
Magnesium Sulfate	0.05 g
Bacto Agar	15 g
Malachite Green	0.001 g

Final pH 6.6 ± 0.2 at 25%C

Middlebrook ADC Enrichment

Formula Per Liter

Albumin Fraction V, Bovine	5 g
Dextrose	2 g
Catalase (Beef)	0.003 g
Distilled water	100 ml

Middlebrook OADC Enrichment

Formula Per Liter

Oleic Acid	0.05 g
Albumin Fraction V, Bovine	5 g
Dextrose	2 g
Catalase (Beef)	0.004 g
Sodium Chloride	0.85 g
Distilled water	100 ml

Middlebrook OADC Enrichment w/WR 1339

Formula Per Liter

Oleic Acid	0.05 g
Albumin Fraction V, Bovine	5 g
Dextrose	2 g
Catalase (Beef)	0.004 g
Sodium Chloride	0.85 g
Distilled water	100 ml
WR 1339, Triton®	0.25 g

Glycerol

Formula Per Liter

Glycerol	100 %

Precautions

1. For Laboratory Use.
2. **Middlebrook 7H9 Broth**
   Middlebrook 7H10 Agar
   Mycobacteria 7H11 Agar

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

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

Storage

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

Store Middlebrook Enrichments at 2-8°C.

Store prepared media at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Middlebrook 7H9 Broth
Middlebrook 7H10 Agar
Mycobacteria 7H11 Agar
Middlebrook ADC Enrichment
Middlebrook OADC Enrichment
Middlebrook OADC Enrichment w/WR 1339
Glycerol

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Tween® 80 (optional)

Method of Preparation

Middlebrook 7H9 Broth

1. Dissolve 4.7 grams in 900 ml of distilled or deionized water containing 2 ml of Glycerol (or 0.5 g Tween® 80, if desired).
2. Autoclave at 121°C for 10 minutes.
3. Cool the medium to 45°C.
4. Aseptically add 100 ml Middlebrook ADC Enrichment. Mix well.

Middlebrook 7H10 Agar

1. Suspend 19 grams in 900 ml of distilled or deionized water containing 5 ml Glycerol.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Cool medium to 50-55°C.
5. Aseptically add 100 ml Middlebrook OADC Enrichment or Middlebrook OADC Enrichment w/WR 1339. Mix well.

Mycobacteria 7H11 Agar

1. Suspend 21 grams in 900 ml distilled or deionized water containing 5 ml glycerol.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 50-55°C.
5. Aseptically add 100 ml Middlebrook OADC Enrichment. Mix well.

Specimen Collection and Preparation[7]

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

Test Procedure

1. Inoculate the specimen onto the medium.
2 Incubate tubes for up to eight weeks.
3 Examine tubes for growth.

Results

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

Limitations of the Procedure

Negative culture results do not rule out active infection by mycobacteria. 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 the mycobacteria;
4. Proper aerobic and increased CO_2 tension were not provided during incubation.

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria: molecular genetic insights. Clin. Microbiol. Rev. **8**:496-514.
2. **Kleitmann, W.** 1995. Resistance and susceptibility testing for *Mycobacterium tuberculosis.* Clin. Microbiol. News. **17**:65-69.
3. **Nolte, F. S., and B. Methcock.** 1995. *Mycobacterium*, p. 400-437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Middlebrook, G., M. L. Cohn, W. B. Dye, W. B. Russell, Jr., and D. Levy.** 1960. Microbiologic procedures of value in tuberculosis. Acta. Tubercul. Scand., **38:**66.
5. **Cohn, M. L., R. F. Waggoner, and J. K. McClatchy.** 1968. The 7H11 Medium for the cultivation of mycobacteria. Am. Rev. Resp. Dis., **98**:295.
6. **Tenover, F. C., J. T. Crawford, R. E. Huebner, L. J. Geiter, C. R. Horsburgh, Jr., and R. C. Good.** 1993. The resurgence of tuberculosis: is your laboratory ready? J. Clin. Microbiol. **31**:767-770.
7. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, sup. 1. American Society for Microbiology, Washington, D.C.
8. Am. Rev. Resp. Dis., **97**:1133.

Packaging

Middlebrook 7H9 Broth	500 g	0713-17
Middlebrook 7H10 Agar	500 g	0627-17
	20 tubes	0627-39
	100 tubes	0627-79
Mycobacteria 7H11 Agar	500 g	0838-17
	20 tubes	0838-39
	100 tubes	0838-79
Middlebrook ADC Enrichment	2 x 20 ml	0714-64
Middlebrook OADC Enrichment	12 x 20 ml	0722-64
	6 x 100 ml	0722-73
Middlebrook OADC Enrichment w/ WR 1339	6 x 20 ml	0801-63
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Milk Agar

Intended Use

Bacto Milk Agar is recommended by the British Standards Institute[1] and the International Dairy Federation for the enumeration of microorganisms in liquid milk, ice cream, dried milk and whey.

Summary and Explanation

Liquid milk is a highly perishable foodstuff with a shelf life of only 5-10 days after pasteurization. Contamination of raw milk may arise from either the soiled or diseased udder or inadequately cleaned milking or storage equipment. Bovine mastitis or udder inflammation may cause contamination with *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* or, more rarely, *Yersinia enterocolitica* and *Leptospira* species. Excretion of these organisms can increase the bulk milk count by 10^5 organisms/ml.

Poor cleaning of the milking equipment may cause contamination with micrococci, streptococci, coliforms or heat resistant *Bacillus* strains, giving an increase of the bulk milk count of >5 x 10^4 organisms/ml. Spoilage of pasteurized or raw milk by proteolytic psychrotrophic bacteria can occur on prolonged storage below 7°C.

Milk Agar conforms to the EEC Commission for the examination of ice cream.[2] Milk Agar is recommended for performing plate count tests on milks, rinse waters, milk products and ice cream.[3]

Principles of Procedure

Tryptone and Yeast Extract provide essential nutrients while Skim Milk Powder is a source of casein. Dextrose is the carbon energy source.

Proteolytic bacteria will be surrounded by a clear zone from the conversion of casein into soluble nitrogenous compounds.[1]

Formula

Milk Agar

Formula Per Liter

Tryptone	5 g
Yeast Extract	2.5 g
Dextrose	1 g
Skim Milk Powder (antibiotic free)	1 g
Bacto Agar	12.5 g

Final pH 6.9 ± 0.1 at 25°C

Precautions

1. For In 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

Milk Agar

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.2% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, clear to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 2.2% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

Prepare medium per label directions. Inoculate using the pour plate technique and incubate at 30°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus casei	9595	30-300	good
Lactococcus lactis	19435	30-300	good
Staphylococcus aureus	25923*	30-300	good
Streptococcus thermophilus	19258	30-300	good

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

Materials Required but not Provided

Flasks with closures
Distilled or deionized water
Hot plate
Autoclave
Dilution tubes containing 1/4-strength Ringer's solution
Petri dishes
1% hydrochloric acid or 10% acetic acid

Method of Preparation

1. Suspend 22 grams in 1 liter distilled or deionized water and boil gently to dissolve completely.
2. Dispense 10-12 ml per tube. Cap loosely.
3. Autoclave at 121°C for 15 minutes.
4. Pour Milk Agar into Petri dishes for the spread plate technique or allow tubes to cool to 45°C for the pour plate technique.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Total counts may be carried out using either pour plates or surface counting techniques.

1. Prepare milk dilutions of 1/10, 1/100, 1/1,000 in 1/4-strength Ringer's solution. Use this inoculum within 15 minutes.
2. **Pour Plates**: Pipette 1 ml of each dilution into Petri dishes. Add 10-12 ml of molten Milk Agar, cooled to 45°C, and mix thoroughly.

   Spread Plates: Spread 1 ml of milk dilution over the surface of the solidified medium in a Petri dish.
3. Incubate at 30°C for 72 hours.

Results

Select plates containing 10-300 colonies. Results are expressed as colonies per ml of product tested.

Proteolytic psychrotrophic colonies may be enhanced by flooding the plates with a solution of 1% hydrochloric acid or 10% acetic acid. Pour off the excess acid solution and count the colonies surrounded by clear zones.

References

1. **Microbiological examination for dairy purposes.** Diluents, media and apparatus and their preparation and sterilisation. BS4285, Sec. 1.2.
2. **Klose, J.** 1968. Susswaren. **14:**778-782.
3. **Dept. of Health.** 1987. Memo.139/Foods.

Packaging

Milk Agar	500 g	1859-17
	5 kg	1859-03

Bacto® Minerals Modified Glutamate Broth

Intended Use

Bacto Minerals Modified Glutamate Broth is used for enumerating coliform organisms in water.

Also Known As

Gray's Minerals Modified Glutamate Broth

Summary and Explanation

Gray[1] described a simple formate-lactose-glutamate medium that could be used as an alternative to MacConkey Broth for the presumptive identification of coliform bacteria in water. Gray's original medium gave fewer false positive results than MacConkey Broth, was suitable for use at 44°C, and gave low volumes of gas.

The medium was improved[2] by the addition of ammonium chloride that, by replacing ammonium lactate, resulted in a doubling of the gas volume. The addition of B complex vitamins, certain amino-acids and magnesium ions resulted in an increased rate of fermentation. Comparative trials of the modified glutamate medium and MacConkey Broth[3] with chlorinated and unchlorinated waters showed that Gray's Minerals Modified Glutamate Broth gave significantly higher numbers of positive results (acid and gas production) for coliform organisms and *Escherichia coli*. This was especially apparent after 48 hours of incubation but was also clearly seen with unchlorinated water samples after only 24 hours incubation. For chlorinated water samples, results with the two media were comparable. After 18-24 hours incubation, Minerals Modified Glutamate Medium gave significantly fewer false positive reactions. *Clostridium perfringens*, a common cause of false positive reactions in MacConkey media, is unable to grow in a minimal-glutamate based medium.

A major feature of Minerals Modified Glutamate Medium is its superiority in initiating growth of *Escherichia coli* after exposure to chlorine when incubated for 48 hours. In view of the known resistance to chlorination of some viruses, the ability to isolate coliform bacteria that survive marginal chlorination provides an additional safety factor in water treatment.

In a comparison to Lauryl Tryptose Lactose Broth[4], Minerals Modified Glutamate Medium gave superior isolation of *Escherichia coli* after 48 hours incubation by the multiple tube method, especially in waters containing small numbers of organisms. Minerals Modified Glutamate Medium is the medium of choice for the detection of fecal contamination in chlorinated drinking water supplies in Great Britain.[4]

Abbiss et al.[5] compared Minerals Modified Glutamate Medium and three other enrichment broths for the enumeration of coliform organisms present in soft cheese, cooked meat and patè. Minerals Modified Glutamate Medium was superior in sensitivity to Lauryl Sulfate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth.

Minerals Modified Glutamate Broth has been used in the modified direct plate method for enumeration of *Escherichia coli* biotype 1 in foods.[6] According to this method, 15 grams of agar are added per liter of single strength broth before autoclaving. The medium is poured in 12-15 ml amounts into sterile Petri dishes. This resuscitation agar is used for the recovery of damaged cells from frozen or dried foodstuffs.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige, free flowing, homogeneous.
Solution:	1.77% solution, soluble in distilled or deionized water on gentle warming. Solution is purple, clear.
Reaction of 1.77% Solution at 25°C:	pH 6.7 ± 0.1

(containing 0.25 grams of ammonium chloride per 100 ml)

Cultural Response

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

ORGANISM	ATCC®	CFU	GROWTH/GAS PRODUCTION
Enterobacter aerogenes	13048*	30-300	Good growth and turbidity Acid (yellow) production with gas
Enterococcus faecalis	19433*	1,000	No visible growth
Escherichia coli	25922*	30-300	Good growth and turbidity Acid (yellow) production with gas
Salmonella typhimurium	14028*	30-300	Good growth and turbidity. Negative for acid and gas.

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.

Principles of the Procedure

Sodium Glutamate and Sodium Formate are the basis of a defined minimal medium for the enumeration of coliform organisms in water. Lactose is the carbohydrate source in Minerals Modified Glutamate Broth. The addition of B complex vitamins, certain amino acids, and magnesium ions allows an increased rate of fermentation. Phosphate acts as a buffering agent. The addition of Ammonium Chloride allows increased gas production by the test organism. Bromocresol Purple is present as a pH indicator.

Formula

Minerals Modified Glutamate Broth

Formula Per Liter

Sodium Glutamate	6.4 g
Lactose	10 g
Sodium Formate	0.25 g
L-cystine	0.02 g
L(-) Aspartic Acid	0.024 g
L(+) Arginine	0.02 g
Thiamine	0.001 g
Nicotinic Acid	0.001 g
Pantothenic Acid	0.001 g
Magnesium Sulfate Heptahydrate	0.1 g
Ferric Ammonium Citrate	0.01 g
Calcium Chloride Dihydrate	0.01 g
Dipotassium Phosphate	0.9 g
Bromocresol Purple	0.01 g

Final Ph 6.7 ± 0.1 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Minerals Modified Glutamate Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Ammonium chloride
Bunsen burner or magnetic hot plate
Test tubes with closures
Fermentation vials
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 17.7 grams in distilled or deionized water, the amount of water depending on the strength of medium desired:
   Single-strength medium - 1 liter of water;
   Double-strength medium - 500 ml of water.
2. Add 2.5 grams of ammonium chloride per liter and mix well.
3. Heat gently to dissolve completely.
4. Dispense into tubes as listed below and place an inverted fermentation vial into each tube:
   Double strength medium - 1 x 5 ml;
   Double strength medium - 5 x 10 ml;
   Single strength medium - 10 x 5 ml.
5. Autoclave at 115-116°C for 10 minutes. Before opening the autoclave, allow the temperature to drop below 75°C to avoid entrapped air bubbles in the inverted fermentation vials.

Specimen Collection and Preparation

Follow laboratory procedure for specimen collection.

Test Procedure

The multiple tube method is used for the enumeration of *Escherichia coli* and coliform organisms using Minerals Modified Glutamate Broth.

For good quality water, inoculate the water sample into the medium in the following volumes:

1. 50 ml of sample into 50 ml of double-strength medium;
2. 5 x 10 ml of sample into 5 x 10 ml of double-strength medium.

For more polluted waters, inoculate the water sample into the medium in the following volumes:

1. 5 x 1 ml of sample into 5 x 5 ml of single-strength medium;
2. 5 x 1 ml of a 1:10 dilution of the sample into 5 x 5 ml of single-strength medium.

Incubate the tubes at 35 ± 2°C. Examine after 18-24 hours incubation and again at 48 hours,

Results

All tubes demonstrating acid production, indicated by the medium turning yellow, and gas, either in the inverted fermentation vial or by effervescence on shaking, may be regarded as presumptive positive reactions. Each presumptive positive tube should be confirmed in Brilliant Green Bile 2%, as well as with additional biochemical tests.

The most probable number of organisms in 100 ml of the original water sample can be calculated using the following table.[7]

Quantity of Water in Each Tube	50 ml	10 ml	Most Probable Number (MPN)
Number of Tubes Used	1	2	of coliforms in 100 ml in sample
	0	0	0
	0	1	1
	0	2	2
Number of Tubes	0	3	4
Giving Positive	0	4	5
Reaction	0	5	7
	1	0	2
	1	1	3
	1	2	6
	1	3	9
	1	4	16
	1	5	+18

Limitations of the Procedure

1. The performance of the medium is significantly affected by pH. Avoid overheating the broth. Check the pH of each lot before proceeding with testing.
2. Due to the nutritional requirements of the organisms, some organisms other than coliform bacteria may grow in the medium with production of acid and gas. Test all presumptive-positive tubes to confirm the presence of *Escherichia coli*.

References

1. **Gray, R. D.** 1959. Formate Lactose Glutamate: A chemically defined medium as a possible substitute for MacConkey Broth in the presumptive coliform examination of water. J. Hyg., Camb. **57:**249-265.
2. **Gray, R. D.** 1964. An improved formate-lactose-glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water. J. Hyg., Camb. **62:**495-508.
3. **P. H. L. S. Standing Committee on the Bacteriological Examination of Water Supplies.** 1968. Comparison of MacConkey Broth, Teepol Broth and Glutamic Acid Media for the enumeration of coliform organisms in water. J. Hyg., Camb. **65:**67-82.
4. **Joint Committee of the P. H. L. S. and the Standing Committee of Analysts.** 1980. A comparison between Minerals Modified Glutamate Medium and Lauryl Tryptose Lactose Broth for the enumeration of *Escherichia coli* and coliform organisms in water by the multiple tube method. J. Hyg., Camb. **85:**35-48.

5. **Abbiss, J. S., J. M. Wilson, R. M. Blood, and B. Jarvis.** 1981. A comparison of Minerals Modified Glutamate Medium with other media for the enumeration of coliforms in delicatessen foods. J. Appl. Bact. **51:**121-127.
6. **Holbrook, R., J. M. Anderson, and A. C. Baird-Parker.** 1980. Modified Direct Plate Method for counting *Escherichia coli* in foods. Food Technol. in Aust. **32:**78- 83.
7. **Departments of the Environment, Health & Social Security, and P.H.L.S.** 1982. The Bacteriological Examination of Drinking Water Supplies, Report on Public Health and Medical Subjects No. 71. HMSO, London.

Packaging

Minerals Modified Glutamate Broth	500 g	1850-17

Bacto® Minimal Agar Davis
Bacto Minimal Broth Davis w/o Dextrose

User Quality Control

Identity Specifications

Minimal Agar Davis

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.66% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, very slightly to slightly opalescent.
Prepared Medium:	Medium amber, very slightly to slightly opalescent.
Reaction of 2.66% Solution at 25°C:	pH 7.0 ± 0.2

Minimal Broth Davis w/o Dextrose

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	1.06% solution, soluble in distilled or deionized water. Solution is colorless, clear.
Prepared Medium:	Colorless, clear.
Reaction of 1.06% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Minimal Agar Davis

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	6883	100-1,000	good
Escherichia coli	9637	100-1,000	good

Minimal Broth Davis w/o Dextrose

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis	6633	100-1,000	good
Escherichia coli	6883	100-1,000	good
Escherichia coli	9637	100-1,000	good

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

Intended Use

Bacto Minimal Agar Davis is used for isolating and characterizing nutritional mutants of *Escherichia coli*.

Bacto Minimal Broth Davis w/o Dextrose is used with added dextrose in isolating and characterizing nutritional mutants of *Escherichia coli* and *Bacillus subtilis*.

Summary and Explanation

Lederberg[1] described the Davis formulation for Minimal Agar Davis. Minimal Broth Davis w/o Dextrose is the same formulation without dextrose and agar. Both media support the growth of nutritional mutants of *E. coli* while Minimal Broth Davis w/o Dextrose with added dextrose also supports the growth of nutritional mutants of *B. subtilis*.

Lederberg[1] described two techniques for isolating nutritional mutants of *E. coli*, one by random isolation and the other by delayed enrichment. Both Lederberg[1] and Davis[2] described a third technique using penicillin. Nutritional mutants of *B. subtilis* can be isolated by these three techniques and by a modification of the penicillin technique described by Nester, Schafer and Lederberg.[3]

After the mutants are isolated, they are characterized biochemically by growth in minimal broth supplemented with specific growth factors or groups of growth factors. It is generally best to classify mutants according to their requirements for amino acids, vitamins, nucleic acids or other substances. This is done by supplementing the minimal medium with Vitamin Assay Casamino Acids plus tryptophane, or a mixture of water soluble vitamins, alkaline-hydrolyzed yeast, nucleic acid or yeast extract, depending on the particular mutants desired. The supplemented minimal broth is inoculated with a slightly turbid suspension of the mutant colonies and incubated for 24 hours at 35°C. Growth with Vitamin Assay Casamino Acids indicates a vitamin requirement. When a major growth factor group response in obtained, the characterization is carried further by the same general procedure to subgroups and finally to individual growth substances.

Principles of the Procedure

Minimal Agar Davis and Minimal Broth Davis w/o Dextrose contain citrate and phosphates as buffers. Ammonium Sulfate is the carbon source. Magnesium is a cofactor for many metabolic reactions. Minimal Agar Davis contains Dextrose as the carbohydrate energy source; Bacto Agar is the solidifying agent.

Formula

Minimal Agar Davis

Formula Per Liter

Bacto Dextrose	1 g
Dipotassium Phosphate	7 g
Monopotassium Phosphate	2 g
Sodium Citrate	0.5 g
Magnesium Sulfate	0.1 g
Ammonium Sulfate	1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Minimal Broth Davis w/o Dextrose

Formula Per Liter

Dipotassium Phosphate	7 g
Monopotassium Phosphate	2 g
Sodium Citrate	0.5 g
Magnesium Sulfate	0.1 g
Ammonium Sulfate	1 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Minimal Broth Davis w/o Dextrose**

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

   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

Minimal Agar Davis
Minimal Broth Davis w/o Dextrose

Materials Required but not Provided

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

Method of Preparation

Minimal Agar Davis

1. Suspend 26.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Minimal Broth Davis w/o Dextrose

1. Dissolve 10.6 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Cool to room temperature.
4. Aseptically add 10 ml 10% dextrose solution at room temperature.
5. Mix thoroughly.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Random Technique

1. Irradiate a cell suspension of wild type *E. coli.*
2. Dilute the suspension 100-500 times.
3. Culture on a complete agar medium containing all the necessary growth requirements.
4. Incubate the cultures at 35°C for 24 hours.
5. Select isolated colonies and inoculate into Minimal Broth Davis and a nutritionally complete broth.
6. Incubate at 35°C for 24 hours.
7. Observe growth in both media.

Delayed Enrichment Method

1. Prepare plates of Minimal Agar Davis by pouring a 15-20 ml base layer in a 95 mm sterile Petri dish followed by a 5 ml seed layer.
2. Inoculate with a diluted irradiated *E. coli* suspension.
3. Pour a 5-10 ml layer of uninoculated Minimal Agar Davis over the seed layer.
4. Incubate for 24 hours or longer to allow for the growth of prototroph cells (wild type cells).
5. Pour a layer of a complete agar medium over the minimal agar medium to develop the mutant cells.
6. Incubate at 35°C for 6-12 hours.

Penicillin Method

1. Wash an irradiated *E. coli* suspension with sterile saline and dilute to 20 times the original volume in sterile minimal broth.
2. Dispense into tubes in desired amounts.
3. Add freshly prepared penicillin to each tube to give a final concentration of 200 units per ml.
4. Incubate at 35°C for 4-24 hours on a shaker.
5. Spread 0.1 ml, 0.01 ml and 0.001 ml samples onto complete agar plates.
6. Incubate at 35°C for 24 hours.
7. Select isolated colonies and test for growth in minimal broth.

***Bacillus subtilis* Procedure**

1. Grow cultures of *Bacillus subtilis* in Antibiotic Medium 3 for 18 hours.
2. Centrifuge to sediment the cells.
3. Aseptically decant the supernatant fluid.

4. Resuspend the cells in minimal medium and centrifuge.
5. Decant the supernatant and resuspend the pellet in minimal medium to give a cell concentration of about 2 x 10^8 cells per ml.
6. Irradiate the suspension with a low pressure mercury ultraviolet lamp for a sufficient time to give a cell survival of 1 x 10^4 cells per ml.
7. Incubate the suspension at room temperature for 4-18 hours in the minimal medium with appropriate substances added to allow for the growth of desired mutants.
8. Wash the culture in sterile minimal medium.
9. Centrifuge and resuspend in the same medium.
10. Dilute 1 to 10 with sterile minimal medium.
11. Let stand for 60 minutes to starve the mutants.
12. Add penicillin to give a concentration of 2,000 units per ml.
13. Incubate 15 minutes.
14. Plate the culture on nutrient agar for colony isolation.
15. Identify the nutrition mutants by transferring colonies by replicate plating onto plates of minimal agar which has been supplemented with the appropriate nutritional substances.

Results

Random Technique

Growth in the nutritionally complete medium and no growth in the Minimal Broth indicates a mutant.

Delayed Enrichment Method

Mutant colonies will grow as small colonies after the addition of the complete medium which diffuses through the Minimal Agar.

Penicillin Method

Mutant colonies grow after the addition of penicillin.

***B. subtilis* Method**

Mutant colonies grow on Nutrient Agar after the addition of penicillin.

Limitations of the Procedure

1. Strains vary in their sensitivity to penicillin. Adjustments to the time of treatment and concentration of penicillin may be necessary.[1]

References

1. **Lederberg, J.** 1950. Isolation and characterization of biochemical mutants of bacteria. Methods in Med. Res. **3**:5-21.
2. **Davis.** 1949. Proc. Nat'l Acad. Sci. **35**:1.
3. **Nester, Schafer, and Lederberg.** 1963. Genetics **48**:529.

Packaging

Minimal Agar Davis	500 g	0544-17
Minimal Broth Davis w/o Dextrose	500 g	0756-17

Bacto® Mitis Salivarius Agar
Bacto Chapman Tellurite Solution 1%

Intended Use

Bacto Mitis Salivarius Agar is used with Bacto Chapman Tellurite Solution 1% in isolating *Streptococcus mitis*, *S. salivarius* and enterococci, particularly from grossly contaminated specimens.

Summary and Explanation

Streptococcus mitis, *Streptococcus salivarius* and *Enterococcus* species are part of the normal human flora. *S. mitis* and *S. salivarius* are known as viridans streptococci. These organisms play a role in cariogenesis and infective endocarditis and cause an increasing number of bacteremias.[1] Enterococci cause urinary tract infections, wound infections and bacteremia.[2] These organisms can colonize the skin and mucous membranes.

Chapman[3,4,5] investigated methods for isolating streptococci and formulated Mitis Salivarius Agar. The medium facilitates isolation of *S. mitis* (*Streptococcus viridans*), *S. salivarius* (non-hemolytic streptococci) and enterococci from mixed cultures.[6]

Principles of the Procedure

Mitis Salivarius Agar contains Tryptose, Proteose Peptone No. 3 and Proteose Peptone as sources of carbon, nitrogen, vitamins and minerals. Dextrose and Saccharose are carbohydrate sources. Crystal Violet and Potassium Tellurite (from Chapman Tellurite Solution 1%) inhibit most gram-negative bacilli and most gram-positive bacteria except streptococci. Trypan Blue gives the colonies a blue color. Bacto Agar is the solidifying agent.

Formula

Mitis Salivarius Agar

Formula Per Liter

Bacto Tryptose	10 g
Bacto Proteose Peptone No. 3	5 g
Bacto Proteose Peptone	5 g
Bacto Dextrose	1 g
Bacto Saccharose	50 g
Dipotassium Phosphate	4 g
Trypan Blue	0.075 g
Bacto Crystal Violet	0.0008 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Chapman Tellurite Solution 1%

Sterile 1% solution of Potassium Tellurite

Precautions

1. **Mitis Salivarius Agar**: For Laboratory Use.
   Chapman Tellurite Solution 1%: For Laboratory Use.
2. **Chapman Tellurite Solution 1%**

   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist.

Wear suitable protective clothing. Keep container tightly closed.

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

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

Storage

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

Store Chapman Tellurite Solution 1% 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

Mitis Salivarius Agar
Chapman Tellurite Solution 1%

Materials Required but not Provided

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

Method of Preparation

1. Suspend 90 grams of Mitis Salivarius Agar in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Just prior to dispensing, add 1 ml Chapman Tellurite Solution 1%.
5. DO NOT HEAT THE COMPLETE MEDIUM.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

See appropriate references for specific procedures.

Results

S. mitis produces small or minute blue colonies. These colonies may become easier to distinguish with longer incubation. *S. salivarius* produces blue, smooth or rough "gum drop" colonies, 1-5 mm in diameter depending on the number of colonies on the plate. *Enterococcus* species form dark blue or black, shiny, slightly raised, 1-2 mm colonies.

Limitations of the Procedure

1. If coliforms grown on the medium, they produce brown colonies.

User Quality Control

Identity Specifications

Mitis Salivarius Agar

Dehydrated Appearance:	Bluish beige, free-flowing, homogeneous.
Solution:	9.0% solution, soluble in distilled or deionized water on boiling. Solution is deep royal blue, very slightly opalescent.
Prepared Medium:	Deep royal blue, slightly opalescent.
Reaction of 9.0% Solution at 25°C:	pH 7.0 ± 0.2

Chapman Tellurite Solution 1%

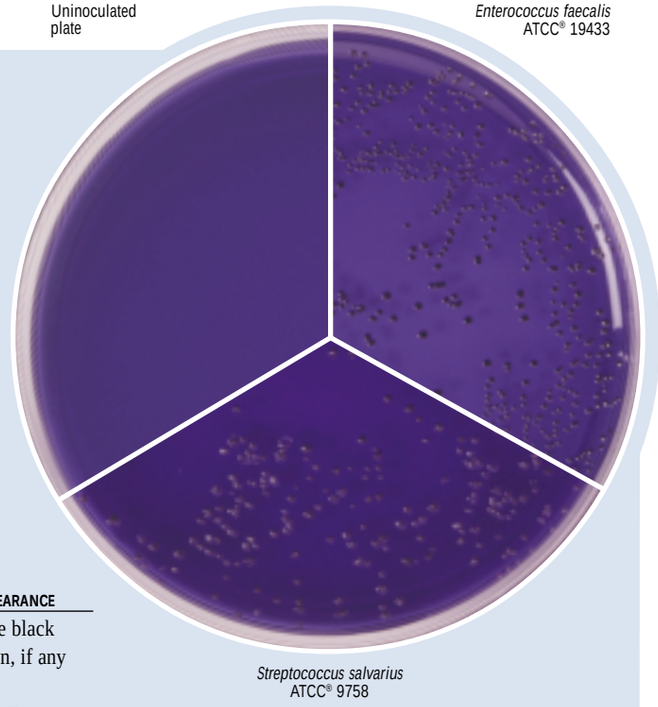
Appearance:	Colorless, clear, may have a slight precipitate.

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	19433	100-1,000	good	blue black
Escherichia coli	25922*	1,000-2,000	partial to complete inhibition	brown, if any
Staphylococcus aureus	25923*	1,000-2,000	partial to complete inhibition	–
Streptococcus mitis	9895	100-1,000	good	blue
Streptococcus salivarius	9758	100-1,000	good	blue "gum drop" shape

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



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

2. Molds will grow on the medium after two days incubation.
3. *Erysipelothrix rhusiopathiae* produces colorless, circular, convex colonies.
4. Beta-hemolytic streptococci produce colonies that resemble *S. mitis*.

References

1. **Facklam, R. R., and J. A. Washington II.** 1991. *Streptococcus* and related catalase-negative gram-positive cocci. p. 238-257. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology. Washington, D.C.
2. **Facklam, R.R., and D. F. Sahm.** 1995. *Enterococcus*, p. 308-314. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Chapman, G. H.** 1944. The isolation of streptococci from mixed cultures. J. Bacteriol. **48**:113.
4. **Chapman, G. H.** 1946. The isolation and testing of fecal streptococci. Am. J. Dig. Dis. **13**:105.
5. **Chapman, G. H.** 1947. Relationship of nonhemolytic and viridans streptococci in man. Trans. N.Y. Acad. Sci. (Series 2) **10**:45.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 522-526. Williams & Wilkins, Baltimore, MD.

Packaging

Mitis Salivarius Agar	500 g	0298-17
Chapman Tellurite Solution 1%	6 x 1 ml	0299-51
	6 x 25 ml	0299-66

Bacto® Modified EC Medium
Bacto Novobiocin Antimicrobic Supplement

Intended Use

Bacto Modified EC Medium is used with Bacto Novobiocin Antimicrobic Supplement in the detection of *Escherichia coli* O157:H7 in meat and poultry products.

User Quality Control

Identity Specifications

Modified EC Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.66% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear, with no significant precipitate.
Reaction of 3.66% Solution at 25°C:	pH 6.9 ± 0.2

Novobiocin Antimicrobic Supplement

Lyophilized Appearance:	White cake.
Rehydrated Appearance:	Colorless solution.

Cultural Response

Prepare Modified EC Medium per label directions. Add 10 ml of Novobiocin Antimicrobic Supplement per liter. Inoculate the tubes and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	33186	1,000-2,000	none to poor
Escherichia coli O157:H7	35150	10-100	good

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

Also Known As

mEC+n or Modified *E. coli* Medium

Summary and Explanation

Modified EC Medium and Novobiocin Antimicrobic Supplement are based on the formula for modified EC broth with novobiocin (mEC+n) as described by Okrend and Rose.[1] In modifying the EC Medium formula, Okrend and Rose reduced the Bile Salts No. 3 from 1.5 grams per liter to 1.12 grams per liter and added 20 milligrams per liter of sodium novobiocin. Okrend, Rose *et al*. reported that mEC+n was useful in the enrichment and detection of *E. coli* O157:H7 from meats and poultry products.[2-4]

Principles of the Procedure

Tryptone supports good growth of *E. coli* O157:H7 and is rich in peptides and nitrogen. Lactose is an additional source of carbon for organisms, such as *E. coli*, that can ferment this sugar. Potassium Phosphate Dibasic and Monobasic are buffers that facilitate recovery of injured cells. Sodium Chloride provides a suitable ionic environment for growth of microorganisms.

Selectivity of the medium is achieved by the incorporation of Bile Salts No. 3 into the base medium and by the addition of Sodium Novobiocin to the complete medium. These agents suppress the growth of nuisance organisms commonly found in foods. The Sodium Novobiocin is provided in the freeze-dried state as Novobiocin Antimicrobic Supplement. This supplement is rehydrated before use with sterile distilled or deionized water.

Formula

Modified EC Medium

Formula per liter

Bacto Tryptone ..... 20 g

Bacto Bile Salts No. 3	1.12	g
Bacto Lactose	5	g
Potassium Phosphate, Dibasic	4	g
Potassium Phosphate, Monobasic	1.5	g
Sodium Chloride	5	g

Final pH 6.9 ± 0.2 at 25°C

Novobiocin Antimicrobic Supplement

Formula per 10 ml vial

Sodium Novobiocin	20	mg

Precautions

1. For Laboratory Use.
2. **Modified EC Medium**

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

   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.

   Novobiocin Antimicrobic Supplement

   HARMFUL. HARMFUL BY INHALATION AND IF SWALLOWED. (EC) MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. 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 container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

1. Store Modified EC Medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.
2. Store Novobiocin Antimicrobic Supplement at 2-8°C.
3. 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

Modified EC Medium

Novobiocin Antimicrobic Supplement

Materials Required But Not Provided

MacConkey Sorbitol Agar
EMB Agar
Flasks with closures
Sterile distilled or deionized water
Autoclave
Incubator (35°C)
Incubator (42°C)
Butterfield's Phosphate Diluent
Phenol Red Sorbitol Agar with MUG

Method of Preparation

Rehydrate Novobiocin Antimicrobic Supplement with 10 ml sterile distilled or deionized water.

1. Dissolve 36.6 grams Modified EC Medium in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Cool to room temperature.
4. Aseptically add 10 ml rehydrated supplement to 1 liter sterile basal medium. Mix well.

Specimen Collection and Preparation

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

Test Procedure

Many procedures and systems have been described for the use of Modified EC Medium with Novobiocin in the selective and differential enrichment of *E. coli* O157:H7 in meat and poultry samples. Please consult appropriate references.[4-6] Listed below is the USDA's recommended procedure for the enrichment and detection of *E. coli* O157:H7 in meat and poultry samples using Modified EC Medium with Novobiocin.[2-4]

1. Inoculate 25 grams of meat sample into 225 ml of Modified EC Medium with Novobiocin in a stomacher bag. Blend or stomach as required (i.e., 2 minutes) for thorough mixing.
2. Incubate at 35°C for 24 hours.
3. Dilute cultures 10-fold in Butterfield's Phosphate Diluent and inoculate 0.1 ml of appropriate dilutions using a spread plate technique onto MacConkey Sorbitol Agar (MSA) and MacConkey Sorbitol Agar with BCIG (MSA-BCIG) agar plates.
4. Incubate plates at 42°C for 24 hours.
5. Examine MSA plates for sorbitol-negative colonies (white) and MSA-BCIG plates for sorbitol-negative, BCIG-negative colonies (white).
6. Subculture sorbitol-negative colonies to respective plates of EMB Agar and Phenol Red Sorbitol Agar containing MUG (PRS-MUG).
7. Incubate EMB and PRS-MUG Agar plates at 35°C for 18-24 hours. Examine plates for sorbitol fermentation, MUG reaction (fluorescence), and typical *E. coli* growth on EMB Agar.

Results

Growth in Modified EC Medium with Novobiocin is demonstrated as an increase in turbidity. Colonies of *E. coli* O157:H7 appear white on MacConkey Sorbitol and MacConkey Sorbitol-BCIG Agars. Fermentation of sorbitol in Phenol Red Sorbitol Broth is demonstrated by the production of a yellow color in the medium. With sorbitol non-fermenters, the color of the medium remains red to reddish purple. Positive MUG reactions are demonstrated as a blue fluorescence in the medium under long-wave UV light. Colonies of *E. coli* on EMB Agar appear blue-black to dark purple. A green metallic sheen may also be present.

Cultures that are sorbitol-negative, MUG-negative, and produce blue-black to dark purple colonies with a green metallic sheen on EMB Agar are indicative of *E. coli* O157:H7. These cultures should be tested serologically and with additional biochemical testing to confirm their identity as *E. coli* O157:H7.

Limitations

1. Nutritional requirements may vary from strain to strain.

References

1. **Okrend, A. J. G., and B. E. Rose.** 1989. Isolation and identification of *E. coli* O157:H7 from meat. USDA Food Safety Inspection Service. Rev. 3 of Laboratory Communication no. 38. *E. coli* O157:H7. 20 December 1989. U.S. Department of Agriculture, Washington, D.C.
2. **Okrend, A. J. G., B. E. Rose, and B. Bennett.** 1990. A screening method for the isolation of *E. coli* O157:H7 from ground beef. J. Food Prot. **53:**249-252.
3. **Okrend, A. J. G., B. E. Rose, and C. P. Lattuada.** 1990. Use of 5-bromo-4-chloro-3-indoxyl-ß-D-glucuronide in MacConkey Sorbitol Agar to aid in the isolation of *E. coli* O157:H7 from ground beef. J. Food Prot. **53:**941-943.
4. **Okrend, A. J. G., B. E. Rose, and R. Matner.** 1990. An improved screening method for the detection and isolation of *E. coli* O157:H7 from meat, incorporating the 3M Petrifilm Test Kit-HEC-for hemorrhagic *Escherichia coli* O157:H7. J. Food Prot. **53:**936-940.
5. **Hawkins, E. W., and L. E. Orme.** 1995. Rapid testing methodology for *Escherichia coli* O157:H7 using commercially available products. Proc. West. Sec., Amer. Soc. Animal Sci. vol. 46.
6. **Johnson, R. P., R. J. Durham, S. T. Johnson, and L. A. MacDonald.** 1995. Detection of *E. coli* O157:H7 in meat by an enzyme-linked immunosorbent assay, EHEC-Tek. Appl. Environ. Microbiol. **61:**386-388.

Packaging

Modified EC Medium	500 g	0340-17
Novobiocin Antimicrobic Supplement	6x10 ml	3197-60*

*Store at 2-8°C.

Bacto® Modified Letheen Agar
Bacto Modified Letheen Broth

Intended Use

Bacto Modified Letheen Agar and Bacto Modified Letheen Broth are used for the microbiological testing of cosmetics.

Summary and Explanation

Modified Letheen Agar and Modified Letheen Broth are based on Letheen Agar, Modified and Letheen Broth, Modified as described in the 7th edition of the U.S. FDA Bacteriological Analytical Manual.[1] Letheen Agar, Modified and Letheen Broth, Modified are recommended by the FDA for use in the microbiological testing of cosmetics.[2]

Principles of the Procedure

Beef Extract and Tryptone provide carbon and nitrogen sources required for good growth of a wide variety of bacteria and fungi. The Tryptone level was increased in the Modified Letheen Agar and Broth formulas to provide for better growth. Vitamins and cofactors, required for growth as well as additional sources of nitrogen and carbon, are provided by Yeast Extract. Sodium Chloride provides a suitable osmotic environment. In Modified Letheen Broth, Sodium Chloride is provided by the Letheen Broth component. Both media also contain polysorbate 80, lecithin and sodium bisulfite to partially neutralize the preservative systems commonly found in cosmetics. Bacto Agar is included in Modified Letheen Agar as a solidifying agent.

Formula

Modified Letheen Agar

Formula Per Liter

Bacto Letheen Agar	32 g
Bacto Tryptone	5 g
Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	2 g
Sodium Chloride	5 g
Sodium Bisulfite	0.1 g
Bacto Agar	5 g

Final pH 7.2 ± 0.2 at 25°C

Modified Letheen Broth

Formula Per Liter

Bacto Letheen Broth	26.7 g
Bacto Tryptone	5 g
Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	2 g
Sodium Bisulfite	0.1 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

Storage

Store the dehydrated 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.

Procedure

Materials Provided

Modified Letheen Agar
Modified Letheen Broth

User Quality Control

Identity Specifications

Modified Letheen Agar

Dehydrated Appearance:	Tan, homogeneous, appears moist with a tendency to clump.
% Solution:	5.91% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, after cooling in approx. 45-50°C waterbath, clear to slightly opalescent, may have slight fine precipitate.
Prepared Medium:	Light-medium amber, slightly opalescent, may have a slight fine precipitate.
Reaction of 5.91% Solution at 25°C:	pH 7.2 ± 0.2

Modified Letheen Broth

Dehydrated Appearance:	Tan, homogeneous, appears moist with a tendency to clump.
% Solution:	4.38% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, after cooling, clear to slightly opalescent, may have slight fine precipitate.
Prepared Medium:	Medium-dark amber after cooling, slightly opalescent, may have slight fine precipitate.
Reaction of 4.38% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Modified Letheen Agar or Broth per label directions. Inoculate and incubate at 35°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH (AGAR/BROTH)
Staphylococcus aureus	6538	25-100	Good

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

Materials Required But Not Provided

Glassware
Autoclave
Incubators (35°C, 30°C)
Selective media

Method of Preparation

1. Suspend the medium in 1 liter of distilled or deionized water:
   Modified Letheen Agar - 59.1 grams;
   Modified Letheen Broth - 43.8 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense as desired.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure [2]

1. Prepare and dilute samples in Modified Letheen Broth in accordance with established guidelines.
2. Using the spread plate technique, inoculate in duplicate 0.1 ml of the diluted samples onto Modified Letheen Agar, Potato Dextrose Agar (or Malt Extract Agar) containing chlortetracycline, Baird Parker Agar (or Vogel-Johnson Agar, optional), Anaerobic Agar, and a second set of Modified Letheen Agar plates.
3. Incubate one set of Modified Letheen Agar plates at 30 ± 2°C for 48 hours and the other set at 35 ± 2°C under anaerobic conditions for 2-4 days. Incubate the Potato Dextrose Agar (or Malt Extract Agar) plates at 30 ± 2°C for 7 days and the Baird Parker Agar (or Vogel-Johnson Agar) plates, if inoculated, at 35 ± 2°C for 48 hours.
4. Incubate the diluted samples from step 1 at 35 ± 2°C for 7 days. Subculture enriched samples onto Modified Letheen Agar only if there is no growth on the primary Modified Letheen Agar plates.

Results

Examine plates for evidence of growth and characteristic colonial morphology. Determine colony counts and subculture each colony type onto Modified Letheen Agar and MacConkey Agar (also Baird Parker or Vogel-Johnson Agar, if used in step 2).

Determine Gram reaction, cell morphology and catalase reactions. Identify bacterial isolates in accordance with established procedures.[2]

References

1. **Tomlinson, L. (ed.).** 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
2. **Hitchins, A. D, T. T. Tran, and J. E. McCarron**. 1992. *In* L.A. Tomlinson (ed.), FDA Bacteriological Analytical Manual, 7th Ed. AOAC International, Arlington, VA.

Packaging

Modified Letheen Agar	500 g	0631-17-0
Modified Letheen Broth	500 g	0630-17-0

Bacto® Modified Listeria Enrichment Broth

Intended Use

Bacto Modified Listeria Enrichment Broth is used for selectively enriching *Listeria* from raw and pasteurized milk according to the International Dairy Federation.[1]

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[2] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[3] The first reported food-borne outbreak of listeriosis was in 1985.[4] Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[5]

Implicated vehicles of transmission include turkey frankfurters,[6] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants. It is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[7]

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 Tubes:	Light yellowish-amber, clear to very slightly opalescent.
Reaction of 3.61% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	29212*	2,000-10,000	partially suppressed at 18- 24 hours
Escherichia coli	25922*	2,000-10,000	marked to complete inhibition
Listeria monocytogenes	19114	100-1,000	good
Saccharomyces pastorianus	9080	2,000-10,000	marked to complete inhibition

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.

Listeria species grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside these parameters.[8] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C. Many common food contaminants such as streptococci, enterococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* interfere with the isolation of *Listeria monocytogenes*.[9]

Listeria Enrichment Broth is based on the formula developed by Lovett et al.[10] in which Tryptic Soy Broth was supplemented with yeast extract for optimum growth of *Listeria*. Modified Listeria Enrichment Broth is a modification of Listeria Enrichment Broth in which the acriflavine content has been reduced from 15 mg to 10 mg per liter. This modification reflects the lower concentration specified by the International Dairy Federation[1] for isolation of *L. monocytogenes* from milk and milk products.

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

Principles of the Procedure

Modified Listeria Enrichment Broth contains Tryptone, Soytone and Yeast Extract as nitrogen and vitamin sources. Dextrose provides an energy source. Sodium Chloride maintains the osmotic balance of the medium. Potassium Phosphate is a buffering agent. Cycloheximide is incorporated to inhibit saprophytic fungi, while Nalidixic Acid inhibits growth of gram-negative organisms. Acriflavine HCl is added at 10 mg per liter to suppress growth of gram-positive bacteria.

Formula

Modified Listeria Enrichment Broth

Formula Per Liter

Bacto Tryptone	17 g
Bacto Soytone	3 g
Dextrose	2.5 g
Sodium Chloride	5 g
Potassium Phosphate, Dibasic	2.5 g
Bacto Yeast Extract	6 g
Cycloheximide	0.05 g
Acriflavine HCl	0.01 g
Nalidixic Acid	0.04 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **TOXIC.** HARMFUL BY INHALATION AND IF SWALLOWED. (EC) MAY CAUSE CANCER. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Do not breathe dust. In case of accident or if you feel unwell, seek medical advice immediately. (Show label where possible.) Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Cardiovascular, Face, Lungs, Nerves, Skin, Thorax.

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

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

Storage

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

Modified 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

1. Suspend 36.1 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[1]
2. For specific information about sample preparation and inoculation, consult appropriate reference.[1]

Test Procedure

For dairy samples, the IDF[1] selective enrichment method is as follows:

1. Add 25 ml liquid or 25 grams of solid test material to 225 ml Modified Listeria Enrichment Broth and mix or blend thoroughly.
2. Incubate for 48 hours at 30°C.[1]
3. At 48 hours, streak the Modified Listeria Enrichment Broth culture onto plates of Oxford Medium or Palcam Medium.
4. Incubate the agar plates at 37°C for 48 ± 2 hrs.

Results

1. Examine agar plates for typical *Listeria* colonies.
2. Consult appropriate references for selection of biochemical or serological tests for confirmation of *Listeria* sp.[1,8,11,12]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains of *Listeria* may be encountered that fail to grow or grow poorly on this medium.
2. Modified Listeria Enrichment Broth is a partially selective medium. Growth of some contaminating strains will be markedly but not totally inhibited.

References

1. **International Dairy Federation.** 1990. Milk and milk products - detection of *Listeria monocytogenes*. IDF Provisional International Standard No. 143. International Dairy Federation, Brussels.
2. **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.
3. **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.
4. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma Special Report. J. Assoc. Off. Anal. Chem. **70**:769-772.
5. **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.
6. **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.
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.
8. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*, p. 637-663. *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.
9. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
10. **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.
11. **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. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
12. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products. *In* R. T. Marshall (ed.). Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Modified Listeria Enrichment Broth	500 g	0205-17
	10 kg	0205-08

Bacto® Motility GI Medium

Intended Use

Bacto Motility GI Medium is used for detecting motility of microorganisms and for separating organisms in their motile phase.

Summary and Explanation

Motility GI Medium is prepared according to the formulation of Jordan, Caldwell and Reiter.[1] It is a semisolid gelatin-heart infusion medium that is adaptable to use in both tubes and plates for motility studies.

Principles of the Procedure

Heart Infusion Broth and Gelatin provide nitrogen, vitamins, and amino acids. Bacto Agar is the solidifying agent. Motility is evidenced by the presence of diffuse growth away from the line or spot of inoculation. Nonmotile organisms grow only along the line of inoculation.

Formula

Motility GI Medium

Formula Per Liter

Bacto Heart Infusion Broth	25 g
Bacto Gelatin	53.4 g
Bacto Agar	3 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

Storage

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

Store the prepared medium 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

Motility GI Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (50-55°C)

Method of Preparation

1. Suspend 81.4 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. If tubes are desired, dispense medium to a depth of 60-75 mm.
4. Autoclave at 121°C for 15 minutes.
5. Cool tubes by placing in cold water up to the depth of the medium. Cool flasks of medium to 50-55°C; pour into sterile Petri dishes to a depth of 1/8 inch or more and allow to solidify.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	8.14% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, very slightly opalescent, without precipitate.
Prepared Medium:	Medium amber, slightly opalescent.
Reaction of 8.14% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	GROWTH	MOTILITY
Escherichia coli	25922*	good	+
Klebsiella pneumoniae	13883*	good	–

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

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

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Klebsiella pneumoniae* ATCC® 13883

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate with growth from an 18-24 hour pure culture.
2. If tubes are used, inoculate by stab inoculation. If plates are used, spot the inoculum on the surface or stab just below the medium surface.
3. Incubate at a temperature and duration appropriate for the suspected organism being tested.
4. Examine tubes or plates for growth and signs of motility.

Results

Motility is evidenced by the presence of diffuse growth away from the line or spot of inoculation. Nonmotile organisms grow only along the line of inoculation.

Limitations of the Procedure

1. All weak or questionable motility test results should be confirmed by flagella stain or by direct wet microscopy.[2]
2. Some flagellar proteins are not synthesized at higher temperatures.[3]
3. Some isolates of *Yersinia enterocolitica* demonstrate motility at 35°C while others may be nonmotile at 25°C.[2] The motility of *Proteus* is also temperature dependent. This effect of temperature on motility needs to be taken into account when deciding on a testing regimen.
4. Due to the temperature dependency of motility in some organisms, a negative test tube or plate should be incubated an additional 5 days at a lower temperature of 22-25°C.[3]

References

1. **Jordan, E. O., M. E. Caldwell, and D. Reiter.** 1934. Bacterial motility. J. Bacteriol. **27**:165.
2. **D'Amato, R. F., and K. M. Tomfohrde.** 1981. Influence of media on temperature-dependent motility test for *Yersinia enterocolitica*. J. Clin. Microbiol. **14**:347-348.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, MD.

Packaging

Motility GI Medium	500 g	0869-17

Bacto® Motility Medium S

Intended Use

Bacto Motility Medium S is used with Bacto TTC Solution 1% in detecting bacterial motility.

Also Known As

Motility Medium S conforms with Motility S Medium and Motility-Nitrate Medium.

Summary and Explanation

Motility Medium S is prepared according to the formula of Ball and Sellers.[1] The composition of the medium offers no more resistance to motility during incubation than would a broth culture, yet it preserves the stab line. The medium also permits further testing fornitrate reduction, nitrogen gas production and gelatin liquefaction.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, slightly opalescent, without significant precipitate.
Prepared Medium:	Medium amber, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 7.3 ± 0.2

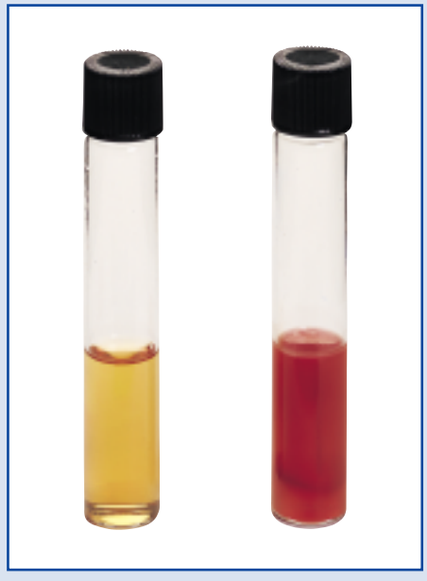
Cultural Response

Prepare Motility Medium S with added TTC Solution 1% per label directions. Stab inoculate the medium and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	MOTILITY	TTC REDUCTION
Escherichia coli	25922*	good	+	+
Klebsiella pneumoniae	13883*	good	–	+
Shigella flexneri	12022	good	–	–

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

*These cultures are available as Bactrol™ disks and should be used according to the technical information.



Uninoculated tube | *Escherichia coli* ATCC® 25922

Principles of the Procedure

Infusion from Beef Heart, Gelatin and Tryptose provide nitrogen, vitamins and amino acids to support growth of fastidious microorganisms. Sodium Chloride maintains the osmotic balance of the medium and also induces the swarming of *Proteus* species.[2] Dipotassium Phosphate provides buffering capability and has been shown to have a stimulatory effect on the motility of *Proteus* species.[1] Potassium Nitrate provides trace elements necessary for bacterial growth. Organisms capable of reducing nitrate, especially nitrate-reducing obligate aerobes[1], exhibit increased motility in the presence of 0.2% Potassium Nitrate. Bacto Agar is a solidifying agent at a concentration that preserves the line of inoculation. TTC (2,3,5-triphenyltetrazolium chloride) facilitates the detection of motility. Growth along or out from the stab line is made readily visible because formazan precipitates when TTC is reduced by a microorganism.

Formula

Motility Medium S

Formula Per Liter	
Beef Heart, Infusion from	500 g
Bacto Tryptose	10 g
Bacto Gelatin	30 g
Sodium Chloride	5 g
Dipotassium Phosphate	2 g
Potassium Nitrate	2 g
Bacto Agar	1 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

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

Storage

Store 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

Motility Medium S

Materials Required But Not Provided

Glassware
Autoclave
Waterbath (60°C)
Refrigerator (2-8°C)
Incubator (35°C)
TTC Solution 1%

Method of Preparation

1. Suspend 60 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 60°C.
4. Aseptically add 10 ml of TTC Solution 1% at 60°C. Mix thoroughly.
5. Dispense into sterile test tubes.
6. Refrigerate at 2-8°C until ready to use.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Stab inoculate with growth from a pure 18-24 hour culture immediately after removing the medium from the refrigerator.
2. Incubate at 35-37°C for 18-48 hours.
3. Observe at 6, 24 and 48 hours.[3] Examine tubes for signs of growth and motility.

Results

Motility is evidenced by the presence of diffuse growth away from the line or spot of inoculation. Nonmotile organisms grow only along the line of inoculation.

Growth of microorganisms capable of reducing TTC will appear as a red color along the stab line as well as in the areas into which the cells have migrated.

Limitations of the Procedure

1. All weak or questionable motility results should be confirmed by flagella stain or by direct wet microscopy.[4]
2. Some flagellar proteins are not synthesized at higher temperatures.[3]
3. Some isolates of *Yersinia enterocolitica* demonstrate motility at 35°C while others may be nonmotile at 25°C.[4] The motility of *Proteus* is also temperature dependent. This affect of temperature on motility needs to be considered when deciding on a testing regimen.
4. Due to the fact that motility is temperature dependent in some organisms, a test tube or plate yielding a negative result should be incubated an additional 5 days at a lower temperature of 22-25°C.[3]
5. The addition of tetrazolium salts to the medium may be inhibitory to some bacteria.[5]

References

1. **Ball, R. J., and W. Sellers.** 1966. Improved motility medium. Appl. Microbiol. **14**:670-673.
2. **Schneierson, S. S.** 1961. Production of discrete nonswarming colonies of *Proteus* on medium deficient in sodium chloride and other salts. J. Bacteriol. **82**:621-622.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
4. **D'Amato, R. F., and K. M. Tomfohrde.** 1981. Influence of media on temperature-dependent motility test for *Yersinia enterocolitica*. J. Clin. Microbiol. **14**:347-348.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Motility Medium S	500 g	0761-17
TTC Solution 1%	30 ml	3112-67*
TTC	25 g	0643-13

*Store at 2-8°C

Bacto® Motility Test Medium

Intended Use

Motility Test Medium is used for detecting microbial motility.

Summary and Explanation

In 1936, Tittsler and Sandholzer reported using a semisolid agar for the detection of bacterial motility.[1] Motility Test Medium is a modification of this formulation.

Bacterial motility is observed macroscopically by a diffuse zone of growth spreading from the line of inoculation. Certain species of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular outgrowths along the stab. Tittsler and Sandholzer reported that tubes incubated for one day gave identical results with the hanging drop method and that incubation for two days permitted them to demonstrate motility in an additional 4% of the cultures tested.[1]

Motility Test Medium is recommended for the detection of microbial motility in food and dairy standard methods.[2,3,4]

Principles of the Procedure

Tryptose is a source of nitrogen, amino acids and carbon. Sodium chloride provides essential ions while maintaining osmotic balance. Agar is a solidifying agent used at a low concentration.

Formula

Motility Test Medium

Formula Per Liter

Bacto Tryptose	10 g
Sodium Chloride	5 g
Bacto Agar	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

Storage

Store the dehydrated medium below 30°C. The 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

Motility Test Medium

Materials Required but not Provided

Glassware
Autoclave
Incubator (35°C)
Incubator (22-25°C)
Inoculating needle
Beef Extract (optional)

Method of Preparation

1. Suspend 20 grams in 1 liter distilled or deionized water. (To obtain more luxuriant microbial growth, add 0.1-0.3% Beef Extract prior to boiling the medium)
2. Heat to boiling to dissolve completely.
3. Dispense as desired.
4. Autoclave at 121°C for 15 minutes.

User Quality Control

Identity Specifications

Dehydrated Media	
Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear to slightly opalescent with no significant precipitate.
Reaction of 2% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Motility Test Medium per label directions. Inoculate by straight stab of the test organisms and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	MOTILITY
Escherichia coli	25922*	good	positive
Klebsiella pneumoniae	13883*	good	negative

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

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

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Klebsiella pneumoniae* ATCC® 13883

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Inoculate tubes with a pure culture by stabbing through the center of the medium with an inoculating needle to approximately one-half the depth of the medium. Incubate at the proper temperature for the organism under consideration and examine at 18-48 hours. If negative, continue incubation at 22-25°C for an additional 5 days.

Results

Motility is manifested macroscopically by a diffuse zone of growth spreading from the line of inoculation. Certain species of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular growths along the stab line. Non-motile organisms grow only along the line of inoculation.

Limitations of the Procedure

1. Many organisms fail to grow deep in semisolid media, inoculating pour plates may be advantageous.[5]

References

1. **Tittsler, R. P., and L. A. Sandholzer.** 1936. The use of semi-solid agar for the detection of bacterial motility. J. Bacteriol. **31**:575-580.
2. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
3. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, p.538-543, vol 1. Williams & Wilkins, Baltimore, MD.

Packaging

Motility Test Medium	100 g	0105-15
	500 g	0105-17

Bacto® Mueller Hinton Medium
Bacto Mueller Hinton Broth

Intended Use

Bacto Mueller Hinton Medium is used for antimicrobial susceptibility testing of rapidly growing aerobic microorganisms by the disk diffusion technique.

Bacto Mueller Hinton Broth is for antimicrobial susceptibility testing of aerobic microorganisms by broth dilution methods.

Also Know As

Mueller Hinton media are abbreviated as M-H Agar and M-H Broth.

Summary and Explanation

Mueller Hinton Medium duplicates the formula recommended by Mueller and Hinton[1] for the primary isolation of *Neisseria* species. In the development of a simple transparent medium containing heat stable ingredients, Mueller and Hinton selected pea meal extract agar.[2] In their modification, starch replaced the growth-promoting properties of pea extract, acting as a "protective colloid" against toxic substances. Tryptic digest of meat was substituted with casamino acids, technical.

Bauer, Kirby, Sherris and Tuck[3] recommended Mueller Hinton Medium for performing antibiotic susceptibility tests using a single disk of high concentration. Mueller Hinton Medium is used in the disk diffusion method of susceptibility testing.[7] Mueller Hinton Broth is used for determining minimal inhibitory concentrations (MICs).[4] Mueller Hinton Medium complies with requirements of the World Health Organization.[8] Mueller Hinton Medium is specified in the FDA Bacteriological Analytical Manual[9] for food testing.

Mueller Hinton Medium is the recommended medium for testing most commonly encountered aerobic and facultatively anaerobic bacteria.[10] This unsupplemented medium has been selected by the National Committee for Clinical Laboratory Standards (NCCLS) for several reasons:[11]

- It shows good batch-to-batch reproducibility.
- It is low in sulfonamide, trimethoprim, and tetracycline inhibitors.
- It gives satisfactory growth of most non-fastidious pathogens.
- A large amount of data has been collected from antimicrobial susceptibility tests with this medium.

A variety of supplements can be added to Mueller Hinton Medium. For testing streptococci, supplementation with 5% defibrinated sheep or horse blood is recommended.[16] GC agar base with added 1% growth supplement, is used for antimicrobial susceptibility testing of *Neisseria gonorrhoeae.* Susceptibility testing of *Haemophilus* species should be performed on *Haemophilus* Test Medium. Mueller Hinton Medium should be supplemented with 2% NaCl for testing methicillin or oxacillin against staphylococci.[5] Mueller Hinton Medium with Rabbit Serum is used for the cultivation and maintenance of *Corynebacterium* species.[6]

Principles of the Procedure

Infusion from Beef and Casamino Acids, Technical provide nitrogen, vitamins, carbon and amino acids in Mueller Hinton media. Starch is added to absorb any toxic metabolites produced. Bacto Agar is the solidifying agent.

The use of a suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and innerzonal growth, is demonstrated on medium possessing high levels of thymidine. The PABA and

thymine/thymidine content of Mueller Hinton Medium and Mueller Hinton Broth are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Formula

Mueller Hinton Medium

Formula Per Liter

Beef, Infusion from	300 g
Bacto Casamino Acids, Technical	17.5 g
Starch	1.5 g
Bacto Agar	17 g

Final pH 7.3 ± 0.1 at 25°C

Mueller Hinton Broth

Formula Per Liter

Beef, Infusion from	300 g
Bacto Casamino Acids, Technical	17.5 g
Bacto Soluble Starch	1.5 g

Final pH 7.3 ± 0.1 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Mueller Hinton Medium
Mueller Hinton Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Sterile Petri dishes
Sterile 5% defibrinated blood (Optional)

User Quality Control

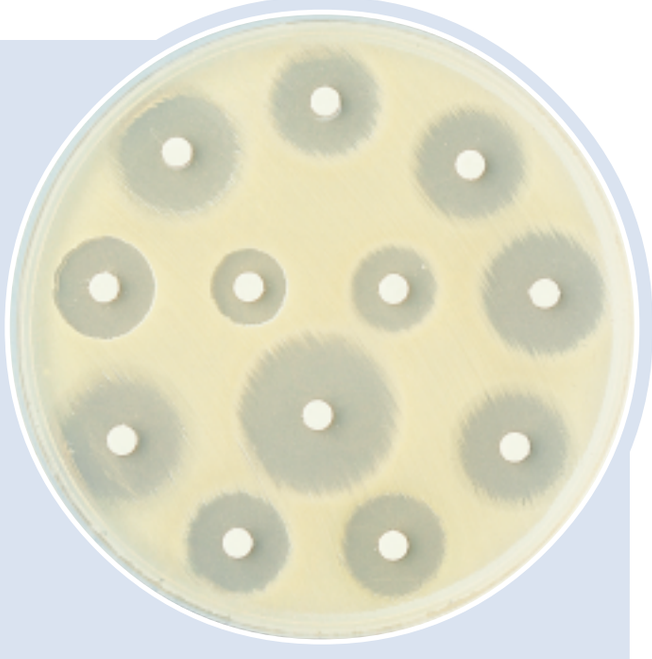
Identity Specification

Mueller Hinton Medium

Dehydrated Appearance:	Beige, homogeneous, free-flowing with few dark specks.
Prepared Medium:	Light to medium amber, slightly opalescent, no significant precipitation.
Reaction of 3.8% Solution at 25°C:	pH 7.3 ± 0.1

Mueller Hinton Broth

Dehydrated Appearance:	Light beige with a few dark specks, homogeneous, free-flowing.
Prepared Medium:	Very light amber, clear, may have slight precipitation.
Reaction of 2.1% Solution at 25°C:	pH 7.3 ± 0.1



Typical test of Mueller Hinton Medium by agar diffusion method.

Cultural Response

Mueller Hinton Medium: Prepare, inoculate and dispense antibiotic disks following the procedure described by NCCLS.[7,11] The cultures listed should have zone sizes near the middle of the range of the concentration tested.[7]

Mueller Hinton Broth: Prepare and dispense into microdilution trays or microdilution tubes described by NCCLS.[4] The cultures listed should have MIC (endpoints) near the middle of the range of the concentration tested.[4]

ORGANISM	ATCC®
Enterococcus faecalis	29212*
Escherichia coli	25922*
Pseudomonas aeruginosa	27853*
Staphylococcus aureus	25923*

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

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

Method of Preparation

Mueller Hinton Medium

1. Suspend 38 g of medium in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. OPTIONAL: Supplement as appropriate.[16] To supplement Mueller Hinton Medium with sheep blood, aseptically add 5% sterile defibrinated blood at 45-50°C. *Haemophilus* Test Medium contains 15 mcg/ml NAD, 15 mcg/ml bovine hematin and 5 mg/ml yeast extract. The medium recommended for testing Neisseria *gonorrhoeae* consists of GC agar base with 10 ml/liter of the following supplement: 1.1 g L-cystine, 0.03 g guanine HCl, 3 mg thiamine HCl, 13 mg PABA, 0.01 g B_{12}, 0.1 g cocarboxylase, 0.25 g NAD, 1 g adenine, 10 g L- glutamine, 100 g glucose, 0.02 g ferric nitrate, 1 l distilled or deionized water.
5. Pour cooled Mueller Hinton Medium into sterile Petri dishes on a level, horizontal surface to give a uniform depth of about 4mm (60 to 70 ml of medium for 150 mm plates and 25 to 30 ml for 100 mm plates) and allow to cool to room temperature.[10]
6. Check prepared Mueller Hinton Medium to ensure the final pH is 7.3 ± 0.1 at 25°C.

Mueller Hinton Broth

1. Suspend 21 g of medium in 1 liter distilled or deionized water.
2. Warm gently to dissolve.
3. Autoclave at 121°C for 15 minutes.
4. Dispense Mueller Hinton Broth into sterile tubes.
5. Check prepared Mueller Hinton Broth to ensure the final pH is 7.3 ± 0.1 at 25°C.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For a complete discussion on antimicrobic susceptibility testing, refer to the appropriate procedures outlined in the references.[4,7,9,10,11,12]

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 these media.
2. Numerous factors can affect results: inoculum size, rate of growth, medium formulation and pH, length of incubation and incubation environment, disk content and drug diffusion rate, and measurement of endpoints. Therefore, strict adherence to protocol is required to ensure reliable results.[12]
3. Disk diffusion susceptibility testing is limited to rapidly growing organisms. Drug inactivation may result from the prolonged incubation times required by slow growers.[12]
4. Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effects of sulfonamides and trimethoprim, causing zones of growth inhibition to be smaller or less distinct.[10]
5. Variation in the concentration of divalent cations, primarily calcium and magnesium, affects results of aminoglycoside, tetracycline, and colistin tests with *P. aeruginosa* isolates.[13,14] A cation content that is too high reduces zones sizes, whereas a cation content that is too low has the opposite effect.[10]
6. When Mueller Hinton Medium is supplemented with blood, the zone of inhibition for oxacillin and methicillin may be 2 to 3 mm smaller than those obtained with unsupplemented agar.[10] Conversely, sheep blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci.[15] Sheep blood may cause indistinct zones or a film of growth within the zones of inhibition around sulfonamide and trimethoprim disks.[10]
7. Mueller Hinton Medium deeper than 4 mm may cause false-resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility report.[10]
8. A pH outside the range of 7.3 ± 0.1 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excessive activity.[10] The opposite effects are possible if the pH is too high.[10]
9. When Mueller Hinton Medium is inoculated, no droplets of moisture should be visible on the surface or on the petri dish cover.[10]
10. Mueller Hinton Medium should be inoculated within 15 minutes after the inoculum suspension has been adjusted.[10]
11. The zone of inhibition diameters of some drugs, such as the aminoglycosides, macrolides, and tetracyclines, are significantly altered by CO_2. Plates should not be incubated in increased CO_2.[10]

References

1. **Mueller, J. H., and J. Hinton.** 1941. A protein-free medium for primary isolation of gonococcus and meningococcus. Proc. Soc. Exp. Biol. Med. **48**:330-333.
2. **Gordon and Hine.** 1916. Br. Med. J. **678**.
3. **Bauer, A. L., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. **45**:493-496.
4. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, PA.
5. **Huang, M., B., E. T. Gay, C. N. Baker, S. N. Banerjee, and F. C. Tenover.** 1993. Two percent sodium chloride is required for susceptibility testing of staphylococci with oxacillin when using agar-based dilution methods. J. Clin. Microbiol. **31**:2683-2688.
6. **Atlas, R. M.** 1993. Handbook of microbiological media. CRC Press, Boca Raton, FL.
7. **National Committee for Clinical Laboratory Standards.** 1993. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A5. National Committee for Clinical Laboratory Standards, Villanova, PA.
8. **World Health Organization.** 1961. Standardization of methods for conducting microbic sensitivity tests. Technical Report Series No. 210, Geneva.
9. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
10. **Wood, G. L., and J. A. Washington.** 1995. Antibacterial susceptibility tests: dilution and disk diffusion methods, p. 1327-1341. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover,

and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

11. **National Committee for Clinical Laboratory Standards.** 1993. Evaluating production lots of dehydrated Mueller-Hinton agar. Tentative standard M6-T. National Committee for Clinical Laboratory Standards, Villanova, PA.
12. **Isenberg, H. E.** (ed.). 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
13. **Barry, A. L., G. H. Miller, C. Thornsberry, R. S. Hare, R. N. Jones, R. R. Lorber, R. Ferraresi, and C. Cramer.** 1987. Influence of cation supplements on activity of netilmicin against *Pseudomonas aeruginosa* in vitro and in vivo. Antimicrob. Agents Chemother. **31**:1514-1518.
14. **Barry, A. L., L. B. Reller, G. H. Miller, J. A. Washington, F. D. Schoenknecht, L. R. Peterson, R. S. Hare, and C. Knapp**. 1992. Revision of standards for adjusting the cation content of Mueller-Hinton broth for testing susceptibility of *Pseudomonas aeruginosa* to aminoglycosides. J. Clin. Microbiol. **30**:585-589.
15. **Buschelman, B. J., R. N. Jones, and M. J. Bale.** 1994. Effects of blood medium supplements on activities of newer cephalosporins tested against enterococci. J. Clin. Microbiol. **32**:565-567.
16. **National Committee for Clinical Laboratory Standards**. 1997. Performance standards for antimicrobial disk susceptibility tests-sixth edition. Approved Standard. M2-A6, Volume 7, No.1 National Committee for Clinical Laboratory Standards, Wayne, PA.

Packaging

Mueller Hinton Broth	100 g	0757-15
	500 g	0757-17
	2 kg	0757-07
Mueller Hinton Medium	100 g	0252-15
	500 g	0252-17
	2 kg	0252-07
	10 kg	0252-08

Bacto® Muller Kauffmann Tetrathionate Broth Base

Intended Use

Bacto Muller Kauffmann Tetrathionate Broth Base is used for enriching *Salmonella* from water, foodstuffs and fecal samples prior to selective isolation.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white to light beige, free-flowing, homogeneous.
Solution:	10.58% solution, insoluble in distilled or deionized water.
Prepared Medium:	Very pale green with white precipitate.

Cultural Response

Prepare Muller Kauffmann Tetrathionate Broth Base per label directions, with the addition of 1.9 ml Iodine solution and 0.95 ml Brilliant Green solution per 100 ml of medium. Inoculate and incubate at 42-43°C for 18-24 hours. Subculture to Brilliant Green Agar. Incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Salmonella typhimurium	14028*	100-1,000	good	red colonies
Salmonella senftenburg (NCTC)	10384	100-1,000	good	red colonies
Escherichia coli	25922*	1,000-2,000	none to poor	–
Proteus vulgaris	13315*	1,000-2,000	none to poor	–

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

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

Summary and Explanation

Muller[1] recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella*. Kauffmann[2] modified the formula to include oxbile and brilliant green as selective agents to suppress bacteria such as *Proteus* spp.

The British Standard Specification specifies Brilliant Green Tetrathionate Broth for isolating *Salmonella* from meat and meat products[3] and from poultry and poultry products.[4] It is also a recommended selective broth for isolating *Salmonella* from animal feces and sewage polluted water.[5] Using more than one selective broth increases the isolation of *Salmonella* from samples with multiple serotypes.[6]

Muller Kauffmann Tetrathionate Broth Base conforms with ISO/DIS 3565.[3]

Principles of the Procedure

Muller Kauffmann Tetrathionate Broth Base contains Bacto Peptone and Beef Extract as sources of carbon, nitrogen, vitamins and minerals. Oxgall and added Brilliant Green are selective agents which inhibit gram positive and other gram negative organisms. Calcium Carbonate is the buffer. Sodium Thiosulfate is a source of sulfur.

Formula

Bacto Muller Kauffmann Tetrathionate Broth Base

Formula Per Liter	
Bacto Beef Extract	5 g
Bacto Peptone	10 g
Sodium Chloride	3 g
Calcium Carbonate	45 g
Sodium Thiosulphate (anhydrous)	38.1 g
Bacto Oxgall	4.7 g

Precautions

1. For Laboratory Use.

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

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Muller Kauffmann Tetrathionate Broth Base

Materials Required but not Provided

Iodine solution (20 g iodine and 25 g potassium iodide in 100 ml water)
Brilliant Green solution (0.1 g Brilliant Green in 100 ml water)
Glassware
Distilled or deionized water
Autoclave
Incubator (43°C)
Buffered Peptone Water
Blender
Tetrathionate Broth
Selenite Brilliant Green Medium
Brilliant Green Agar Enrichment
Brilliant Green Agar

Method of Preparation-Single Strength

1. Suspend 105.8 grams in 1 liter distilled or deionized water.
2. Boil gently.
3. Cool below 45°C.
4. Add 19 ml iodine solution and 9.5 ml brilliant green solution.
5. Dispense into sterile tubes, mixing well to evenly dispense the calcium carbonate.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

Meat and Meat Products

1. Weigh 25 grams of the sample into a sterile blender jar and add 225 ml of Buffered Peptone Water (1810) and macerate for sufficient time to give 10,000-15,000 revolutions.
2. Transfer contents of the blender jar aseptically to a 500 ml flask. Incubate at 37°C ± 0.1°C for 16-20 hours.
3. Transfer 10 ml samples to 100 ml Muller Kauffmann Tetrathionate Broth and to 100 ml Selenite Brilliant Green Medium (0661).
4. Incubate Muller Kauffmann Tetrathionate Broth at 42-43°C and the Selenite Brilliant Green Enrichment at 37°C.
5. Subculture broths after 18-24 hours and 48 hours onto Brilliant Green Agar.
6. Incubate overnight.
7. Examine for the growth of typical colonies of *Salmonella* spp.

Sewage Polluted Natural Waters

This procedure is applicable to the isolation of *Salmonella* spp. other than *S. typhi*.

1. Inoculate 25 ml aliquots of the sample into 25 ml of double strength Buffered Peptone Water (1810). Incubate at 37°C for 18 hours.
2. Transfer 1 ml samples into 10 ml of Muller Kauffmann Tetrathionate Broth.
3. Incubate at 43°C for 48 hours.
4. Subculture broths after 18-24 and 48 hours onto Brilliant Green MacConkey Agar, prepared by adding 10 ml of a 0.33% (w/v) aqueous solution of brilliant green to MacConkey Agar (0331) to give a final concentration of 0.033 g/l.
5. Incubate at 37°C overnight.
6. Examine for colonies typical of *Salmonella* spp.

Results

Salmonella spp. will produce red colonies with good growth.

Limitations of the Procedure

1. The complete medium is unstable and should be used immediately. It may be stored at 2-8°C in the dark for no more than seven days.
2. Due to the nutritional requirements and inhibitory characteristics of the organisms themselves, organisms other than salmonellae, such as *Morganella morganii* and some *Enterobacteriaceae* may grow in the medium.
3. Confirmatory tests, such as fermentation reactions and seroagglutination should be carried out on all presumptive *Salmonella* colonies that are recovered.

References

1. **Muller, L.** 1923. Un nouveau millieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. C. R. Soc. Biol. (Paris) **89**:434-443.
2. **Kauffmann, F.** 1935. Weitere erfahrungen mit dem kombininierten anreicherungsverfahren fur Salmonella bazillen. Ztschr. F. Hyg. **117**:26-32.
3. **International Organization for Standardization.** Geneva. 1974. (Draft International Standard ISO/DIS 3565).
4. A manual for recommended methods for the microbiological examination of poultry and poultry products. 1982.
5. P.H.L.S. Monograph Series No. 8. 1974.
6. **Harvey, R. W. S., and T. H. Price.** 1976. Isolation of salmonellae from sewage- polluted river water using selenite F and Muller-Kauffmann tetrathionate. J. Hyg. Camb. **77**:333-339.

Packaging

Muller Kauffmann Tetrathionate Broth Base	500 g	1853-17

Bacto® Mycobiotic Agar

Intended Use

Bacto Mycobiotic Agar is used for isolating pathogenic fungi.

Summary and Explanation

Numerous media, such as Sabouraud Dextrose Agar, Sabouraud Maltose Agar, Littman Oxgall Agar, Brain Heart Infusion Agar, and Malt Agar have been used widely in culturing pathogenic fungi. The Sabouraud media and Malt Agar are somewhat selective in nature due to low pH, which may suppress bacterial growth. It is well known that media for isolated pathogenic fungi can also be made selective by the addition of antibiotics.[1-8]

Mycobiotic Agar contains cycloheximide and chloramphenicol making it much more selective when compared to other fungal media. This medium has proven useful in the isolation of the dermatophytes and other pathogenic fungi from clinical specimens.[9]

Georg[10] recommends the use of Mycobiotic Agar exclusively for isolating dermatophytes (because none of the dermatophytes are sensitive to cycloheximide or chloramphenicol) and in parallel to media without antibiotics for isolating fungi which cause systemic disease.

Principles of the Procedure

Soytone provides carbon and nitrogen sources. Dextrose is a source of carbon. Cycloheximide suppresses the growth of saprophytic fungi. Chloramphenicol inhibits bacterial growth. Bacto Agar is the solidifying agent.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.56% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent, with no significant precipitate.
Reaction of 3.56% Solution at 25° C:	pH 6.5 ± 0.2

Cultural Response

Prepare Mycobiotic Agar per label directions. Inoculate and incubate at 25-30°C for 18-48 hours. For *Trichophyton mentagrophytes*, inoculate a 1-2 week old undiluted *Trichophyton* culture directly onto a slant or plate. *Trichophyton* cultures should be incubated up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	inhibited
Candida albicans	10231	100-1,000	good
Escherichia coli	25922*	1,000-2,000	inhibited
Trichophyton mentagrophytes	9533	undiluted	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.

Formula

Mycobiotic Agar

Formula Per Liter

Bacto Soytone	10 g
Bacto Dextrose	10 g
Bacto Agar	15 g
Cycloheximide	0.5 g
Chloramphenicol	0.05 g

Final pH 6.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **TOXIC.** TOXIC BY INHALATION AND IF SWALLOWED. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Do not breathe dust. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes/Ears, Blood, Cardiovascular, Lymph Glands, 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, induce vomiting; 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 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

Mycobiotic Agar

Materials Required but not Provided

Glassware
Autoclave
Petri dishes
Tubes with closures

Method of Preparation

1. Suspend 35.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes. Avoid overheating, which will decrease selectivity.

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. Fungi that cause systemic disease may be sensitive to the antibiotics cycloheximide and chloramphenicol. Primary isolation should include the use of both non-selective and selective media.[11] Antibiotic-containing media should be incubated at room temperature. Additional procedures may be required for complete identification of pathogenic yeasts, particularly *Candida albicans*.
2. Although culture techniques are important in the identification of etiological agents of mycotic infections, they are not absolute. Identification must often be accomplished by using direct microscopic examination of the specimen, animal inoculation, biochemical determination, or serological procedures.

References

1. **Leach, B. E., J. H. Ford, and A. J. Whiffen.** 1947. Actidione, an antibiotic from *Streptomyces griseus*. J. Am. Chem. Soc. **69:**474.
2. **Whiffen, A. J.** 1948. The production, assay, and antibiotic activity of actidione, an antibiotic from *Streptomyces griseus*. J. Bact. **56:**283.
3. **Phillips, G. B., and E. Hanel, Jr.** 1950. Control of mold contaminants on solid media by the use of actidione. J. Bacteriology **60:**104-105.
4. **Georg, L. K., L. Ajello, and M. A. Gordon.** 1951. A selective medium for the isolation of *Coccidioides immitis*. Science **114:**387-389.
5. **Fuentes, C. A., F. Trespalacios, G. F. Baquero, and R. Aboulafia.** 1952. Effect of actidione on mold contaminants and on human pathogens. Mycologia **44:**170-175.
6. **Georg.** 1953. Arch. Dermatol. and Syphilol. **67:**355.
7. **Cooke, W. B.** 1954. The use of antibiotics in media for the isolation of fungi from polluted water. Antibiotics and Chemotherapy **4:**657-662.
8. **Robinson, H. M., Jr., M. M. Cohen, R. C. V. Robinson, and E. S. Bereston.** 1956. Simplified office procedures for mycological diagnosis. J. Am. Med. Assoc. **160:**537-540.
9. **Land, G. A.** 1992. Culture media. *In* H. D. Isenberg, (ed.), Clinical microbiology procedures handbook, vol. 1, p. 6.7.1. American Society for Microbiology, Washington, D.C.
10. **Georg, L. K., E. S. McDonough, L. Ajello, and S. Brinkman.** 1960. In vitro effects of antibiotics on yeast phase of *Blastomyces dermatitidis* and other fungi. J. Lab. & Clin. Med. **55:**116-119.
11. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 552-554. Williams & Wilkins, Baltimore, MD.

Packaging

Mycobiotic Agar	100 g	0689-15
	500 g	0689-17
	2 kg	0689-07
	10 kg	0689-08

Mycological Media

Bacto® Mycological Agar · Bacto Mycological Agar w/Low pH

Intended Use

Bacto Mycological Agar is used for cultivating fungi at a neutral pH.

Bacto Mycological Agar w/Low pH is used for isolating and cultivating fungi and aciduric bacteria.

Summary and Explanation

The value of selective media for the initial cultivation of pathogenic fungi has been demonstrated by numerous investigators.[1,2,3] Earlier media for fungi generally relied on an acid pH to make the media less suitable for the growth of many bacteria.[6] More recently developed media use neutral or slightly alkaline reactions,[4,5] antibiotics, bile salts and dyes as selective agents against bacteria.

Mycological media are excellent basal media to which antifungal agents may be added to study their affect on fungi. These media may be supplemented with antibacterial substances to render them more selective for the isolation and cultivation of fungi.

Mycological Agar and Mycological Agar w/Low pH are prepared according to the formulation suggested by Huppert and Walker.[7] Mycological Agar, which has a lower dextrose content than Sabouraud Dextrose Agar, is recommended for the isolation and cultivation of fungi from clinical specimens, foods,[8] and cosmetics.[9] This medium may be adjusted to pH 4.0 after autoclaving by adding sterile lactic acid or acetic acid.

Mycological Agar at a neutral pH is recommended for working with pathogenic fungi. Mycological Agar w/Low pH is suitable for culturing saprophytic yeasts and molds, and aciduric bacteria.

Principles of the Procedure

Soytone provides a source of carbon and nitrogen. Dextrose is an additional source of carbon. Bacto Agar is the solidifying agent.

Formula

Mycological Agar

Formula Per Liter

Bacto Soytone	10 g
Bacto Dextrose	10 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Mycological Agar w/Low pH

Formula Per Liter

Bacto Soytone	10 g
Bacto Dextrose	10 g
Bacto Agar	15 g

Final pH 4.8 ± 0.2 at 25°C

Precautions

1. Mycological Agar: For Laboratory Use.
   Mycological Agar w/Low pH: 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 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

Mycological Agar

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

Mycological Agar w/Low pH

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 4.8 ± 0.2

Cultural Response

Mycological Agar, Mycological Agar w/Low pH

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH MYCOLOGICAL AGAR	GROWTH MYCOLOGICAL AGAR W/LOW PH
Aspergillus niger	16404	100-1,000	good	good
Candida albicans	10231	100-1,000	good	good
Penicillium abeanum	22346	100-1,000	good	good
Saccharomyces carlsbergensis	9080	100-1,000	good	good
Staphylococcus aureus	25923	100-1,000	good	inhibited

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

Procedure

Materials Provided

Mycological Agar
Mycological Agar w/Low pH

Materials Required but not Provided

Glassware
Autoclave
Antibacterial/antifungal agents

Method of Preparation

Mycological Agar, Mycological Agar w/low pH

1. Suspend 35 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. If desired, add antibacterial and antifungal agents after sterilizing and cooling the medium to 45-50°C.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Mycological Agar and Mycological Agar w/Low pH are used in a variety of procedures. Consult appropriate references for further information.[8,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Non-selective fungal media should be used concurrently with selective media when isolating fungi due to the sensitivity of some strains to cycloheximide and chloramphenicol.[10,11,12]

References

1. Am. J. Publ. Health. 1951. **41:**292.
2. Bull. D. Inst. Sieroteropl, Melan. 1926. **5:**173.
3. Am. Rev. Resp. Dis. 1967. **95:**1041.
4. A. J. Clin. Path. 1954. **24:**621.
5. Rev. Latinoam Micobiol. 1958. **1:**125.
6. A. J. Clin. Path. 1951. **21:**684.
7. **Huppert, M., and L. J. Walker.** 1958. The selective and differential effects of cycloheximide on many strains of *Coccidioides immitis*. Am. J. Clin. Pathol. **29:**291.
8. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 552-554. Williams & Wilkins, Baltimore, MD.
9. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr.** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
10. **Georg, L. K., L. Ajello, and C. Papageorge.** 1954. Use of cycloheximide in the selective isolation of fungi pathogenic to man. J. Lab. Clin. Med. **44:**422.
11. **McDonough, E. S., L. Ajello, L. K. Georg, and S. Brinkman.** 1960. *In vitro* effects of antibiotics on yeast phase of *Blastomyces dermatitidis* and other fungi. J. Lab. Clin. Med. **55:**116.

12. **McDonough, E. S., L. K. Georg, L. Ajello, and S. Brinkman.** 1960. Growth of dimorphic human pathogenic fungi on media containing cycloheximide and chloramphenicol. Mycopathol. Mycol. Appl. **13:**113.

Packaging

Mycological Agar	500 g	0405-17
	2 kg	0405-07
Mycological Agar w/Low pH	500 g	0305-17

Bacto® Neopeptone

Intended Use

Bacto Neopeptone is used in preparing microbiological culture media.

Also Known As

Neopeptone is also referred to as Special Peptone.

Summary and Explanation

Neopeptone is particularly well suited to the growth requirements of fastidious miroorganisms. Certain delicate strains of microorganisms are highly susceptible to the effects of bacteriostatic substances frequently present in some peptones. The work of Dubos[1] shows clearly that a peptone free from toxic factors can support the growth of *S. pneumococci* from small inocula. Spray[2] used Neopeptone in his culture media for classification of sporulating anaerobes. Casman[3] reported Neopeptone to be best suited for use in infusion base. Eldering and Kendrick[4] reported good results with Neopeptone in cultivating *Bordetella pertussis*.

Neopeptone is valuable in culture media for the cultivation of pathogenic fungi. Growth of these microorganisms is rapid and colonial formation is uniform and typical for the various types. Bacto Sabouraud Dextrose Agar and Bacto Sabouraud Maltose Agar are prepared with Neopeptone.

Bacto Todd Hewitt Broth prepared with Neopeptone, is excellent for growing Group A streptococci for serological typing. Several media containing Neopeptone are specified in standard methods[5-7] for multiple applications.

User Quality Control

Identity Specifications

Dehydrated Appearance: Tan, free-flowing granules.

Solution: 1%, 2% and 10% solutions are soluble in distilled or deionized water:

1%-Very light to light amber, clear to very slightly opalescent, may have a precipitate.

2%-Light to medium amber, clear to very slightly opalescent, may have a precipitate.

10%-Medium to dark amber, slightly opalescent to opalescent, may have a precipitate.

Reaction of 1% Solution at 25°C: pH 6.9 - 7.5

Cultural Response

All solutions are prepared with the pH adjusted to 7.2 - 7.4.

TEST	SOLUTION	ORGANISM	ATCC®	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	negative
Indole Production	0.1%	*Escherichia coli*	25922*	positive
Acetylmethylcarbinol Production	0.1%	*Enterobacter aerogenes*	13048*	positive
Hydrogen Sulfide	1%	*Salmonella typhi*	6539	positive
Toxicity	2% w/0.5% NaCl & 1.5% Bacto Agar	*Escherichia coli*	25922*	good growth
Toxicity	2% w/0.5% NaCl & 1.5% Bacto Agar	*Staphylococcus aureus*	25923*	good growth

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

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

Principles of the Procedure

Neopeptone is an enzymatic digest of protein. Neopeptone contains a wide variety of peptide sizes in combination with vitamins, nucleotides, minerals and other carbon sources.

Typical Analysis

Physical Characteristics

Ash (%)	7.0	Loss on Drying (%)	3.2
Clarity, 1% Soln (NTU)	1.2	pH, 1% Soln	7.4
Filterability (g/cm^2)	0.3		

Carbohydrate (%)

Total	0.8

Nitrogen Content (%)

Total Nitrogen	13.7	AN/TN (%)	23.8
Amino Nitrogen	3.3		

Amino Acids (%)

Alanine	4.03	Lysine	5.16
Arginine	4.14	Methionine	2.00
Aspartic Acid	6.19	Phenylalanine	8.67
Cystine	0.26	Proline	6.73
Glutamic Acid	13.22	Serine	4.22
Glycine	7.02	Threonine	3.69
Histidine	<0.01	Tryptophan	0.96
Isoleucine	0.36	Tyrosine	4.21
Leucine	3.65	Valine	4.96

Inorganics (%)

Calcium	0.012	Phosphate	2.209
Chloride	0.344	Potassium	0.149
Cobalt	<0.001	Sodium	2.057
Copper	<0.001	Sulfate	0.340
Iron	<0.001	Sulfur	0.657
Lead	<0.001	Tin	<0.001
Magnesium	0.006	Zinc	<0.001
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	2.9
Choline (as Choline Chloride)	3100.0	Pantothenic Acid	16.0
Cyanocobalamin	<0.1	Pyridoxine	2.3
Folic Acid	0.4	Riboflavin	1.3
Inositol	3600.0	Thiamine	<0.1
Nicotinic Acid	52.2	Thymidine	<14.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	400
Salmonella	negative	Thermophile Count	75
Spore Count	175		

Procedure

Materials Provided

Neopeptone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Neopeptone in the formula of the medium being prepared. Add Neopeptone 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 Neopeptone.

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. **Dubos, R.** 1930. The bacteriostatic action of certain components of commercial peptones as affected by conditions of oxidation and reduction. J. Exp. Med. **52**:331-345.
2. **Spray, R. S.** 1936. Semisolid media for cultivation and identification of the sporulating anaerobes. J. Bacteriol. **32**:135.
3. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
4. **Eldering, E., and P. L. Kendrick.** 1936. Some practical considerations in *B. pertussis* vaccine preparation. Am. J. Public Health. **24**:309.
5. **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.
6. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Neopeptone	500 g	0119-17
	10 kg	0119-08

Bacto® Neutralizing Buffer

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous
Solution:	0.52% solution; soluble in distilled or deionized water. Solution is very light to light amber, clear to very slightly opalescent.
Prepared tubes:	Very light to light amber, clear to slightly opalescent without significant precipitation
Reaction of 0.52% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Bacto Neutralizing Buffer per label directions. Dilute a disinfectant containing a quaternary ammonium compound such as Roccal® with Bacto Neutralizing Buffer from 1:2,500 to1:100,000. Inoculate the tubes with *Staphylococcus aureus* ATCC® 6538P. Prepare pour plates by transferring 1 ml from each dilution to Bacto Tryptone Glucose Extract Agar (Product Code 0002). Incubate the plates for 40-48 hours at 32°C. Record growth. Bacto Neutralizing Buffer inactivates the bactericidal activity which the growth pattern should reflect.

Intended Use

Bacto Neutralizing Buffer is recommended for detection of microorganisms found on dairy and food equipment disinfected with chlorine or quaternary ammonium compounds.

Summary And Explanation

Bacto Neutralizing Buffer, a modification of the Standard Methods buffered distilled water, has the ability to inactivate the bactericidal and bacteriostatic effect of chlorine as well as quaternary ammonium compounds. Neutralizing Buffer is recommended for use in the microbiological examination of surfaces in the standard methods for dairy and foods.[1,2] Neutralizing Buffer is also recommended for the digestion and decontamination of mycobacterial specimens.[3]

Principles of the Procedure

Monopotassium phosphate provides the buffering capability. Sodium thiosulfate inactivates the effect of chlorine compounds. The aryl sulfonate complex neutralizes the effects of quaternary ammonium compounds.

Formula

Neutralizing Buffer

Formula Per Liter

Monopotassium Phosphate	0.0425 g
Sodium Thiosulfate	0.16 g
Aryl Sulfonate Complex	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Neutralizing Buffer

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Tryptone Glucose Extract Agar

Method of Preparation

1. Dissolve 5.2 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 standard methods[1,2,3] for specific test methodologies.

Results

Refer to appropriate references and procedures for results.

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Cernoch, P. L., R. K. Enns, M. A. Saubolle, and R. J. Wallace, Jr.** 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coordinating ed., A. S. Weissfeld. American Society for Microbiology, Washington, D.C.

Packaging

Neutralizing Buffer	100 g	0362-15

Bacto® Niacin Assay Medium

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white, homogeneous, tendency to clump.
Solution:	3.75% (single strength) or 7.5% (double strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. Single strength solution is very light amber, clear, may have a slight precipitate.
Prepared Medium:	(Single strength) very light amber, clear, may have a slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Niacin Assay Medium per label directions. Prepare a standard curve using nicotinic acid reference standards at 0.0 to 0.25 µg per 10 ml. The medium supports the growth of *L. plantarum* ATCC® 8014 when supplemented with nicotinic acid.

Intended Use

Bacto Niacin Assay Medium is used for determining niacin concentration by the microbiological assay technique.

Summary and Explanation

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

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

Niacin Assay Medium is prepared according to the formula described by Snell and Wright,[1] modified by Krehl, Strong and Elvehjem[2] and Barton-Wright.[3] Niacin Assay Medium is used in the microbiological assay of nicotinic acid or nicotinamide (niacin) using *Lactobacillus plantarum* ATCC® 8014 as the test organism. The medium complies with USP[4] and AOAC.[5]

Principles of the Procedure

Niacin Assay Medium is a dehydrated medium free from nicotinic acid and its analogs but containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC® 8014. The addition of nicotinic acid or its analogs in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Niacin Assay Medium

Formula Per Liter	
Bacto Vitamin Assay Casamino Acids	12 g
Bacto Dextrose	40 g
Sodium Acetate	20 g
L-Cystine	0.4 g
DL-Tryptophane	0.2 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Thiamine Hydrochloride	200 µg
Calcium Pantothenate	200 µg
Pyridoxine Hydrochloride	400 µg
Riboflavin	400 µg
p-Aminobenzoic Acid	200 µg
Biotin	0.8 µg
Dipotassium Phosphate	1 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Bladder.

   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. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
4. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.
5. 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

Niacin Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus plantarum* ATCC® 8014.
Sterile tubes
Distilled or deionized water
Sterile 0.85% saline
Centrifuge
Spectrophotometer
Lactobacilli Broth AOAC or Micro Inoculum Broth
Niacin

Method of Preparation

1. Suspend 7.5 grams in 100 ml distilled or deionized water.
2. Boil for 2-3 minutes.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volumes to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assay, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Follow assay procedures as outlined in USP[4] and AOAC.[5]

Stock cultures of the test organism *L. plantarum* ATCC® 8014 are prepared by stab inoculation of Lactobacilli Agar AOAC or Micro Assay Culture Agar. After 24-48 hours incubation at 35-37°C, the cultures are kept refrigerated. Transfers are made in triplicate at monthly intervals.

The inoculum for assay is prepared by subculturing a stock culture of *L. plantarum* ATCC® 8014 into 10 ml of Lactobacilli Broth AOAC or Micro Inoculum Broth. After 18-24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions and the supernatant decanted. The cells are washed three times with 10 ml sterile 0.85% saline. After the third wash, the cells are resuspended in 10 ml sterile 0.85% saline and finally diluted 1:100 with 0.85% sterile saline. One drop of this latter suspension is used to inoculate each 10 ml assay tube.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using niacin at levels of 0.0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 µg niacin per assay tube (10 ml). Niacin Assay Medium may be used for both turbidimetric and titrimetric analyses. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C. Titrimetric determinations are best made following 72 hours incubation at 35-37°C.

The concentration of niacin required for the preparation of the standard curve may be prepared by dissolving 0.05 grams of niacin in 1,000 ml

distilled water, giving a stock solution of 50 µg per ml. Dilute the stock solution by adding 1 ml to 999 ml distilled water (50 ng/ml). Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml of the 50 ng/ml solution per tube. Other standard concentrations may be used provided the standard falls within the limits specified by AOAC.[5]

Results

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

Limitations of the Procedure

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

References

1. **Snell and Wright.** 1941. J. Biol. Chem. **13**:675.
2. **Krehl, Strong, and Elvehjem.** 1943. Ind. & Eng. Chem., Ann. Ed., **15**:471.
3. **Barton-Wright.** 1944. J. Biochem. **38**:314.
4. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Inc., Rockville, MD.
5. **Association of Official Analytical Chemists.** 199. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Niacin Assay Medium	100 g	0322-15

Bacto® Nitrate Broth

Intended Use

Bacto Nitrate Broth is used for differentiating microorganisms based on nitrate reduction.

Summary and Explanation

Nitrate reduction is a valuable criterion for differentiating and identifying various types of bacteria. Certain bacteria reduce nitrates to nitrites only, while others are capable of further reducing nitrite to free nitrogen or ammonia.

Nitrites are colorless; however, in an acid environment, they will react with alpha-naphthylamine to produce a pink or red color. When nitrate-positive organisms reduce nitrates to nitrites, a pink color develops in the broth medium when specific reagents are added.

User Quality Control

Identity Specifications

Dehydrated Medium Appearance:	Light to medium tan, free-flowing, homogeneous.
Solution:	0.9% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Reaction of 0.9% Solution at 25°C:	pH 7.0 ± 0.2

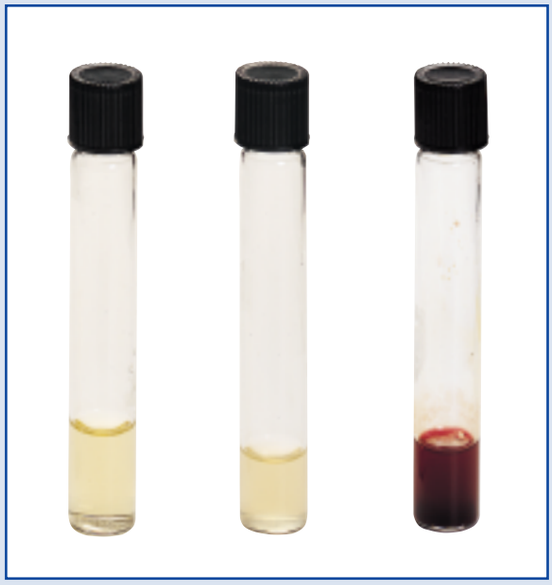
Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	NITRATE REDUCTION
Acinetobacter calcoaceticus	19606	100-1,000	good	–
Enterobacter aerogenes	13048*	100-1,000	good	+
Escherichia coli	25922*	100-1,000	good	+
Salmonella typhimurium	14028*	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.



Uninoculated tube | *Acinetobacter calcoaceticus* ATCC® 19606 | *Escherichia coli* ATCC® 25922

Nitrate-negative organisms, unable to reduce nitrates, yield no color after the reagents are added. Nitrate-negative reactions are tested with zinc dust to confirm the presence of unreduced nitrate.

Principles of the Procedure

Beef Extract and Peptone are sources of carbon, protein and nutrients. Potassium Nitrate is a source of nitrate.

Formula

Nitrate Broth

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Potassium Nitrate	1 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 breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, 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 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

Nitrate Broth

Materials Required but not Provided

Glassware
Autoclave
Incubator (35°C)
0.8% Sulfanilic acid (SpotTest™ Nitrate Reagent A)
N,N-Dimethyl-alpha-naphthylamine (SpotTest Nitrate Reagent B)
Zinc dust (SpotTest Nitrate Reagent C)

Method of Preparation

1. Dissolve 9 grams of Nitrate Broth in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Not applicable

Test Procedure

1. Inoculate the medium with several colonies from a pure 18-24 hour culture. Test an uninoculated control tube in parallel.
2. Incubate the tubes aerobically at 35 ± 2°C for 18-24 hours.
3. Test for nitrate by adding a few drops of 0.8% sulfanilic acid and N,N-dimethyl- alpha-naphthylamine to each tube.
4. Observe for development of a distinct red or pink color within 1-2 minutes, indicating reduction of nitrate to nitrite.
5. If there is no color development, add a pinch of zinc dust (approximately 20 mg on an applicator stick) to the tube. If there is no color development within 5-10 minutes, nitrate was reduced beyond nitrite and the test result is positive.

Results

Development of a distinct red or pink color within 1-2 minutes indicates reduction of nitrate to nitrite and is a positive test result.

Limitations of the Procedure

1. The addition of too much zinc dust may cause a false-negative reaction or a momentary color reaction.[1]
2. The nitrate test is very sensitive. An uninoculated nitrate control should be tested with reagents to determine whether the medium is nitrate free and that the glassware and reagents have not been contaminated with nitrous oxide.[1]
3. The inoculum should not be taken from a liquid or broth suspension of the organism.[1]

References

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

Packaging

Nitrate Broth	500 g	0268-17

Bacto® Nutrient Agar

Intended Use

Bacto Nutrient Agar is used for cultivating a wide variety of microorganisms.

Summary and Explanation

In the early 1900s the American Public Health Association (APHA) suggested this formulation as a standard culture medium for use in bacterial processing for water analysis.[1] The name Nutrient Agar was later adopted for the medium. In Standard Methods of Water Analysis[2] and Standard Methods of Milk Analysis,[3] the APHA advocated the use of dehydrated media for bacterial examination of water and milk. Nutrient Agar meets APHA and Association of Official Analytical Chemists (A0AC) standard methods.[4,5,6]

Nutrient Agar continues to be a widely used general purpose medium for growing nonfastidious microorganisms. It is specified in many standard methods procedures for examining foods, dairy products, water and other materials.[4,5,6,7]

Principles of the Procedure

Nutrient Agar contains Beef Extract and Bacto Peptone as carbon and nitrogen sources for general growth requirements. Bacto Agar is added as a solidifying agent.

Formula

Nutrient Agar

Formula per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use

Storage

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

Expiration Date

The product is stable through the expiration date on the label when stored as directed. Expiration date applies to the medium in its intact container. Do not use if the medium is caked, discolored or shows other signs of deterioration.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in distilled or deionized water on boiling; light to medium amber, clear to slightly opalescent, no significant precipitate.
Prepared medium:	Light amber, very slightly to slightly opalescent, no significant precipitate.
Reaction of 2.3% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	19433*	100-1,000	good
Escherichia coli	25922*	100-1,000	good
Pseudomonas aeruginosa	27853*	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 the Bactrol Disks Technical Information.

Procedure

Materials Provided

Nutrient Agar

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Petri dishes
Incubator

Method of Preparation

1. Suspend 23 grams in 1 liter distilled or deionized water. Heat to boiling to dissolve completely.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Process specimens according to established procedures for the type of material being tested.[4,5,6,7]

Test Procedure

1. Inoculate with growth from either a single colony on agar or a loopful of broth and streak for isolation.
2. Incubate aerobically at 35°C for 18 to 24 hours or longer if necessary.

Results

Good growth of nonfastidious organisms on Nutrient Agar will appear as translucent colonies.

References

1. **American Public Health Association**. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.
2. **American Public Health Association**. 1923. Standard methods of water analysis, 5th ed. American Public Health Association, Washington, D.C.
3. **American Public Health Association**. 1923. Standard methods of milk analysis, 4th ed. American Public Health Association, Washington, D.C.
4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.)**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6. **Association of Official Analytical Chemists**. 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
7. **Vanderzant C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Nutrient Agar	100 g	0001-15
	500 g	0001-17
	2 kg	0001-07

Bacto® Nutrient Agar 1.5%

Intended Use

Bacto Nutrient Agar 1.5% is used for cultivating a variety of microorganisms and with the addition of blood or other enrichment can be used for cultivating fastidious microorganisms.

Summary and Explanation

Nutrient Agar 1.5% is a modification of Nutrient Agar, a formula prepared according to APHA standards.[1,2] This medium is a slightly alkaline general purpose medium. Since this medium contains 0.8% sodium chloride it can be used as a base for enrichment with blood, ascitic fluid or other supplements for cultivating fastidious microorganisms.

Principles of the Procedure

Bacto Beef Extract and Bacto Peptone provide the nitrogen, vitamins, amino acids and carbon sources in Nutrient Agar 1.5%. Sodium chloride maintains the osmotic balance so that red blood cells will not rupture when blood is added as supplement.[1] Bacto Agar is the solidifying agent.

Formula

Nutrient Agar 1.5%

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Sodium Chloride	8 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

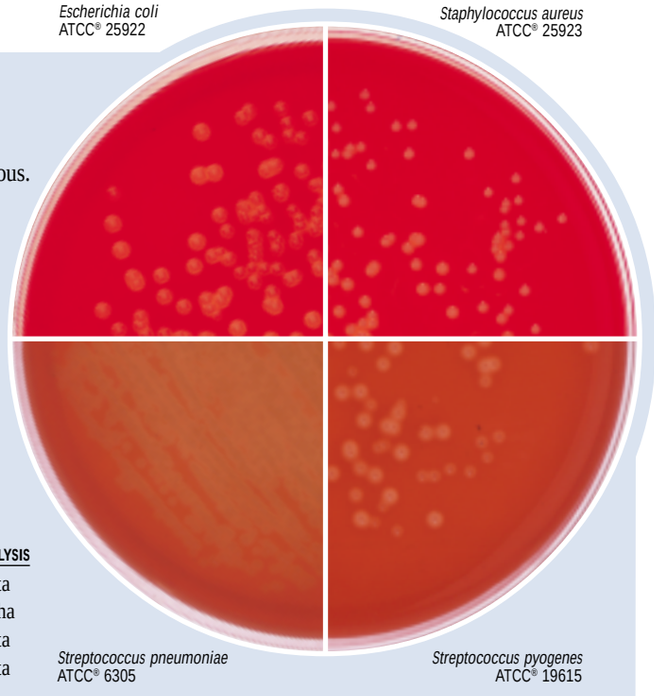
Nutrient Agar 1.5%

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath
Sterile Petri dishes

Method of Preparation

1. Suspend 31 grams in 1 liter distilled or deionized water.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige to light tan, free-flowing, homogeneous.
Solution:	3.1% solution; soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, very slightly to slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Bacto Nutrient Agar 1.5% per label directions.
Inoculate and incubate the plates at 32 ± 1°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH PLAIN	GROWTH W/15% SHEEP BLOOD	HEMOLYSIS
Staphylococcus aureus	25923*	100-1,000	good	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	good	alpha
Streptococcus pyogenes	19615*	100-1,000	good	good	beta
Escherichia coli	25922*	100-1,000	good	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.

2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. To prepared an enriched medium, cool the sterile base to 45-50°C and add the desired enrichment. Mix thoroughly.
5. Dispense 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 aerobic and anaerobic microorganisms, refer to appropriate references.

Results

Refer to appropriate references and procedures for results.

References

1. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Nutrient Agar 1.5%	500 g	0069-17

Bacto® Nutrient Agar with MUG

Intended Use

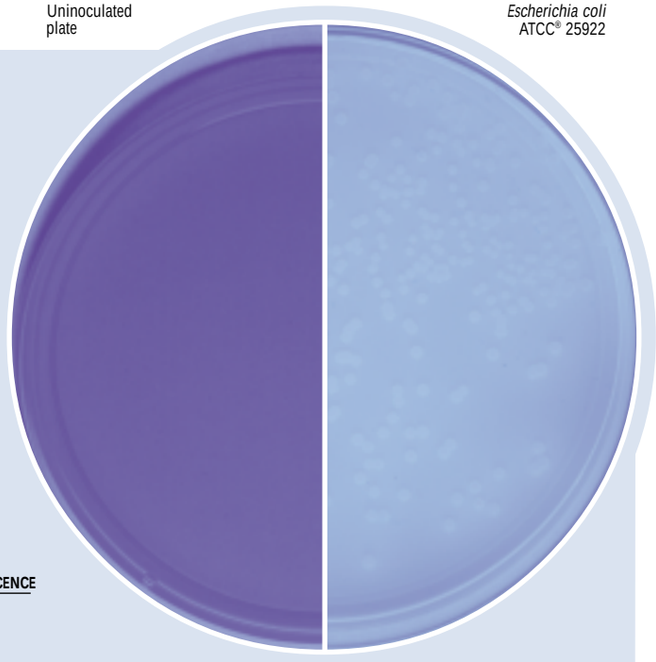
Bacto Nutrient Agar with MUG is used for detecting and enumerating *Escherichia coli* in water.

Summary and Explanation

Escherichia coli is a member of the fecal coliform group of bacteria. The presence of *E. coli* is indicative of fecal contamination.[1] Feng and Hartman[2] developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-ß-D-glucuronide (MUG) at a final concentration of 100 µg/ml into Lauryl Tryptose Broth. Nutrient Agar is similarly modified with the addition of MUG. Rapid quantitation and verification may be achieved with the membrane filtration procedure by transferring the membrane from a total-coliform or fecal-coliform positive sample to a Nutrient Agar substrate containing 4-methylumbelliferyl-ß-D-glucuronide (MUG).[1]

Mates and Shaffer[3] used the membrane filter-Endo Agar method, followed by incubation on Nutrient Agar with MUG, to detect and enumerate *E. coli* within 4 hours of membrane transfer. *E. coli* was recovered at a rate of 98% with no false-positive results.

Nutrient Agar with MUG is prepared according to the formula specified by US EPA[4] and Standard Methods.[1]



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.31% solution, soluble in distilled or deionized water on boiling; light amber, clear to very slightly opalescent.
Prepared Medium:	Light amber, clear to slightly opalescent without significant precipitate.
Reaction of 2.31% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Nutrient Agar with MUG per label directions. After incubation on mEndo Agar LES, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 18-24 hours at 35 ± 2°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH ON mENDO LES	COLONY COLOR	FLUORESCENCE
Enterobacter aerogenes	13048*	30-300	good	red	–
Escherichia coli	25922*	30-300	good	red w/sheen	+

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.

Principles of the Procedure

Beef Extract and Bacto Peptone are sources of nitrogen, vitamins, carbon and amino acids. Bacto Agar is a solidifying agent. The substrate, MUG (4-methylumbelliferyl-ß-D-glucuronide), produces a blue fluorescence when hydrolyzed by the enzyme ß-glucuronidase, which is produced by most *E. coli*.

Formula

Nutrient Agar with MUG

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Agar	15 g
MUG (4-Methylumbelliferyl-ß-D-glucuronide)	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.

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

Nutrient Agar with MUG

Materials Required But Not Provided

Distilled or deionized water
Glassware
Test tubes
Sterile pipettes
Incubator (35°C)
Longwave UV lamp (approximately 366 nm)
mEndo Agar LES
Sterile membranes
Filter apparatus
Petri dishes (50 x 9 mm)
Sterile absorbent pad

Method of Preparation

1. Suspend 23.1 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 into sterile 50 x 9 mm Petri dishes.

Specimen Collection and Preparation

Collect water samples in accordance with recommended procedures.[1]

Test Procedure

Follow the methods and procedures for water testing using mEndo Agar LES in Standard Methods.[1] After incubation on mEndo Agar LES, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 18-24 hours at 35 ± 2°C. Expose the filter surface to longwave UV light.

Results

Observe for fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence around the periphery of the colony under longwave (approximately 366 nm) UV light.

Typical strains of *E. coli* (red with a green metallic sheen on mEndo Agar LES) exhibit blue fluorescence on Nutrient Agar with MUG. Non-*E. coli* coliforms may produce a metallic sheen but do not fluoresce.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Glucuronidase-negative strains of *E. coli* have been encountered.[5,6,7] Similarly, MUG-negative strains of *E. coli* have been reported in this assay procedure but at a very low frequency.[3]
3. Strains of *Salmonella* and *Shigella* species that produce glucuronidase may infrequently be encountered.[8] These strains must be distinguished from *E. coli* on the basis of other parameters, i.e., gas production, lactose fermentation or growth at 44.5°C.

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43**:1320-1329.
3. **Mates, A., and M. Shaffer.** 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriol. **67**:343-346.
4. **Federal Register.** 1991. National primary drinking water regulations; analytical techniques: coliform bacteria. Fed. Regist. **56**:636-643.
5. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of ß-D-glucuronidase-negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.
6. **Hansen, W., and E. Yourassowsky.** 1984. Detection of ß-glucuronidase in lactose fermenting members of the family enterobacteriaceae and its presence in bacterial urine cultures. J. Clin. Microbiol. **20**:1177-1179.
7. **Kilian, M., and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. Acta Pathol. Microbiol. Scand. Sect. B **84**:245-251.
8. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of Escherichia coli in food. J. Food Sc. **50**:1736-1746.

Packaging

Nutrient Agar with MUG	100 g	0023-15
	500 g	0023-17

Bacto® Nutrient Broth

Intended Use

Bacto Nutrient Broth is used for cultivating nonfastidious microorganisms.

Summary and Explanation

In the early 1900s, the American Public Health Association (APHA) suggested this formulation of a standard culture medium for use in bacteriological procedures for water analysis.[1] In *Standard Methods of Water Analysis*[2] and *Standard Methods of Milk Analysis*,[3] the APHA advocated the use of dehydrated culture media for bacteriological examination of water and milk.

Nutrient Broth, a widely used medium, is included in many standard methods procedures. In *Compendium of Methods for the Microbiological Examination of Foods*[4] and *Standard Methods for the Examination of Dairy Products*,[5] Nutrient Broth is included as a satisfactory substitute for the buffered rinse solution used in sampling equipment, containers and air because it effectively neutralizes chlorine and quaternary ammonium compounds. In *Standard Methods for the Examination of Water and Wastewater*, Nutrient Broth is included in testing methods for viruses using microporous filters.[6]

Nutrient Broth is used as a pre-enrichment medium when testing certain 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 and the components of the food sample may hinder growth and recovery of *Salmonella*. Preenrichment in a nonselective medium such as Nutrient Broth allows for repair of cell damage, dilutes toxic or inhibitory substances, and provides a nutritional advantage to *Salmonella* over other bacteria.[4] Nutrient Broth is included in many standard methods procedures for testing foods, dairy products and other materials.[4,5,7,8]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in distilled or deionized water; light to medium amber, clear with no precipitate.
Prepared Medium:	Light to medium amber, clear with no precipitate.
Reaction of 0.8% Solution at 25°C:	pH 6.8 ± 0.2 at 25°C

Cultural Response

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

ORGANISM	ATCC®	CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Staphylococcus aureus	25923*	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 the Bactrol Disks Technical Information.

Principles of the Procedure

Nutrient Broth contains Beef Extract and Bacto Peptone as carbon and nitrogen sources for general growth requirements.

Formula

Nutrient Broth

Formula per liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

Storage

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

Expiration Date

The product is stable through the expiration date on the label when stored as directed. Expiry date applies to the medium in its intact container. Do not use if the medium is caked, discolored or shows other signs of deterioration.

Procedure

Material Provided

Nutrient Broth

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Containers, 225 ml
Incubator

Method of Preparation

1. Dissolve 8 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Test Procedure

Direct:

1. Inoculate the broth with specimen on a swab.
2. Incubate for 18-24 hours at 35 ± 2°C.

As a preenrichment medium when testing certain foods and dairy products for *Salmonella*, consult appropriate references for specific recommendations:[4,5,7,8]

1. Mix 25 grams of sample with 225 ml of Nutrient Broth.
2. Incubate for 18-24 hours at 35 ± 2°C.
3. Transfer a portion to one or more selective enrichment broths.

Results

Turbidity indicates growth.

References

1. **American Public Health Association**. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.

2. **American Public Health Association**. 1923. Standard methods of water analysis, 5th ed. American Public Health Association, Washington, D.C.
3. **American Public Health Association**. 1923. Standard methods of milk analysis, 4th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
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. **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.
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.

Packaging

Nutrient Broth	100 g	0003-15-0
	500 g	0003-17-8
	2 kg	0003-07-0
	10 kg	0003-08-9

Bacto® Nutrient Gelatin

Intended Use

Bacto Nutrient Gelatin is used for detecting gelatin liquefaction by proteolytic microorganisms.

Summary and Explanation

Gelatin was the first gelling agent used to solidify culture media. The advantages of a solid medium were the ability to perform plate counts and to isolate microorganisms in pure culture. The disadvantages of gelatin were the limitation of incubation at 20°C, a temperature that is lower than the optimum for growing many microorganisms, and the fact that many organisms metabolize (liquefy) gelatin. Agar later replaced gelatin as a solidifying agent.

Characterizing fermentative and non-fermentative gram-negative bacilli includes the test for gelatin liquefaction. If the proteolytic enzyme gelatinase is present, gelatin is hydrolyzed and loses its gelling characteristic.[1] Edwards and Ewing include this test in the differentiation scheme for the *Enterobacteriaceae*.[2] Procedures for performing the standard tube method for gelatin liquefaction are available.[2,3,4]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, fine granular.
Solution:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 12.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Nutrient Gelatin per label directions. Using a heavy inoculum, inoculate by stabbing the tube and incubate at 35 ± 2°C for 18-48 hours or up to two weeks, if required. To read gelatinase, refrigerate until well chilled and compare to uninoculated tube. Tilt tubes carefully to test for liquefaction. Tubes positive for gelatinase remain liquid.

ORGANISM	ATCC®	RECOVERY	GELATINASE
Escherichia coli	25922*	good	–
Pseudomonas aeruginosa	27853	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.

Principles of the Procedure

Nutrient Gelatin contains Bacto Peptone and Beef Extract as carbon and nitrogen sources for general growth requirements. Gelatin is the substrate for determining if the microorganism has the proteolytic enzyme to hydrolyze (liquefy) gelatin.

Formula

Nutrient Gelatin

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Gelatin	120 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

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

Storage

Store the dehydrated 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

Nutrient Gelatin

Materials Required but not Provided

Flask with closure
Test tubes with caps
Distilled or deionized water
Autoclave
Incubator (35°C)
Inoculating needle
Refrigerator

Method of Preparation

1. Suspend 128 grams in 1 liter distilled or deionized water.
2. Warm to 50-55°C to dissolve completely.
3. Dispense required amount in test tubes and place caps on tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[1]

1. Using a sterile inoculating needle, touch several similar, well-isolated colonies on agar and stab directly down the center of the tube to approximately 10 mm from the bottom.
2. Incubate at 35 ± 2°C for 24-48 hours. Incubate an uninoculated control tube with the test. Incubation may be extended to 14 days for some organisms.
3. Examine at various intervals.
   a. Transfer the tubes to a refrigerator or ice bath.
   b. Do not shake the tubes when transferring from incubator to refrigerator.
   c. Gently invert the chilled tubes to test for solidity.

Results

Positive: Medium remains liquefied after refrigeration.
Negative: Medium becomes solid after refrigeration.
Uninoculated control tube: Medium becomes solid after refrigeration.

Limitations of the Procedure

1. Use this method for detecting gelatinase only if the identification procedure allows for incubation beyond 48 hours.
2. Gelatin is liquid at temperatures above 20°C. If tubes are incubated at 35°C, they must be refrigerated in order to read for liquefaction. Include an uninoculated tube in the test procedure for comparison.
3. Growth and liquefaction frequently occur only at the surface of the tube. To prevent a false-negative interpretation, handle tubes carefully when warm so that liquefied gelatin remains at the surface of the tube.

References

1. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, sup. 1. American Society for Microbiology, Washington, D.C.
2. **Ewing, W. H.** 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co. Inc., New York, NY.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.)**. 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Nutrient Gelatin	500 g	0011-17

Bacto® NZCYM Broth
Bacto NZYM Broth, Bacto NZM Broth

Intended Use

Bacto NZCYM Broth, Bacto NZYM Broth, and Bacto NZM Broth are used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

NZCYM Broth was developed by Blattner et al. as an enriched medium for cultivating recombinant strains of *E. coli* and propagating λ bacteriophage.[1] *E. coli* grows rapidly in rich media, such as the NZ media, which provide amino acids, vitamins and other metabolites the cell would otherwise have to synthesize.[2] Casein is supplied as Casein Digest in NZ media, a different form than in the LB formulations, providing a good nitrogen source.

The three variations of NZ Media allow the user to select a formulation appropriate to the need.

Principles of the Procedure

Casein Digest, Yeast Extract and Casamino Acids provide the necessary nutrients and cofactors required for excellent growth of recombinant strains of *E. coli*. Due to its higher degree of digestion, Casamino Acids is an excellent source of free amino acids. Sodium Chloride is included in the medium to provide a suitable osmotic environment. Magnesium Sulfate is a source of magnesium ions required in a variety of enzymatic reactions, including DNA replication.

Formula

NZCYM Broth

Formula Per Liter

Bacto Casein Digest	10 g
Bacto Casamino Acids	1 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g
Magnesium Sulfate, Anhydrous	0.98 g

Final pH 7.0 ± 0.2 at 25°C

NZYM Broth

Formula Per Liter

Bacto Casein Digest	10 g
Bacto Yeast Extract	5 g

Sodium Chloride . . . . . 5 g
Magnesium Sulfate, Anhydrous . . . . . 0.98 g
Final pH 7.0 ± 0.2 at 25°C

NZM Broth

Formula Per Liter
Bacto Casein Digest . . . . . 10 g
Sodium Chloride . . . . . 5 g
Magnesium Chloride . . . . . 0.94 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.

User Quality Control

Identity Specifications

NZCYM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.2% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Tubes:	Light to medium amber, clear.
Reaction of 2.2% Solution at 25°C:	pH 7.0 ± 0.2

NZYM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Tubes:	Light to medium amber, clear.
Reaction of 2.1% Solution at 25°C:	pH 7.0 ± 0.2

NZM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.6% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Tubes:	Light to medium amber, clear.
Reaction of 1.6% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

NZCYM Broth, NZYM Broth or NZM Broth

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (C600)	23724	100-300	Good

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.

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

NZCYM Broth
NZYM Broth
NZM Broth

Materials Required But Not Provided

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

Method of Preparation

1. Dissolve the medium in 1 liter of distilled or deionized water:
   NZCYM Broth - 22 grams;
   NZYM Broth - 21 grams;
   NZM Broth - 16 grams.
2. Dispense into tubes with closures.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult an appropriate reference for recommended test procedures.[3]

Results

Growth should be evident in the form of turbidity.

References

1. **Blattner, F. R., B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, and O. Smithies.** 1977. Charon phages: Safer derivatives of bacteriophage λ for DNA cloning. Science **196:**161.
2. **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, vol. 1. Current Protocols, New York, NY.
3. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Packaging

NZCYM Broth	500 g	0404-17
NZYM Broth	500 g	0415-17
NZM Broth	500 g	0435-17

Bacto® OF Basal Medium

Intended Use

Bacto OF Basal Medium is used with added carbohydrate for differentiating gram-negative microorganisms based on oxidation-fermentation patterns.

Also Known As

OF Basal Medium conforms with Oxidation Fermentation Basal Medium.

Summary and Explanation

OF Basal Medium is based on the formula of Hugh and Leifson[1], who described the taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by gram-negative bacteria. When an organism is inoculated into two tubes of OF Basal Medium containing a carbohydrate and the medium in one tube is covered with petrolatum to exclude oxygen, reactions of differential significance may be observed. Fermentative organisms will produce an acid reaction in both the covered and uncovered tubes. Oxidative organisms produce an acid reaction in the uncovered tube while there is little or no growth and no acid formation in the covered tube. Non-oxidative, non-fermentative organisms yield no change in the covered tube and an alkaline reaction in the uncovered tube. OF Basal Medium is listed in standard procedures for the differentiation of gram-negative bacteria based on the oxidation and fermentation of carbohydrates.[2,3,4,5,6]

A modification of OF Basal Medium was developed by Knapp and Holmes[7] to detect acid production from carbohydrates by *Neisseria* spp. and *Branhamella catarrhalis*. The authors reduced the protein concentration relative to the carbohydrate concentration, substituted phenol red for brom thymol blue at a low concentration, and adjusted the initial pH to 7.2. A selective and differential medium for *Pseudomonas cepacia*, based on OF Basal Medium, was developed by Welch et al.[8] by adding agar-agar, lactose, polymyxin B and bacitracin.

Principles of the Procedure

Tryptone provides nitrogen, vitamins and minerals. A high concentration of carbohydrate is added to OF Basal Medium. This helps avoid utilization of peptone by an aerobic organism, which would produce an alkaline reaction and neutralize the slight acidity produced by oxidation.[9] Glucose is the carbohydrate commonly added to the OF Basal Medium; lactose, maltose, mannitol, saccharose and xylose may also be used. Sodium Chloride maintains the osmotic balance of the medium and enhances growth of *Brucella* species.[1] Dipotassium Phosphate provides buffering capacity. Brom Thymol Blue acts as a pH indicator, changing to yellow under acidic conditions. Bacto Agar aids in the determination of motility and also helps evenly distribute any acid produced at the surface of the medium.

Formula

OF Basal Medium

Formula Per Liter

Bacto Tryptone	2 g
Sodium Chloride	5 g
Dipotassium Phosphate	0.3 g
Bacto Brom Thymol Blue	0.08 g
Bacto Agar	2 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige with green tinge, homogeneous, free-flowing.
Solution:	0.94% solution, soluble on boiling in distilled or deionized water. Solution is green, clear, with no precipitate.
Prepared Medium :	Green, clear to very slightly opalescent, with no precipitate.
Reaction of 0.94% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

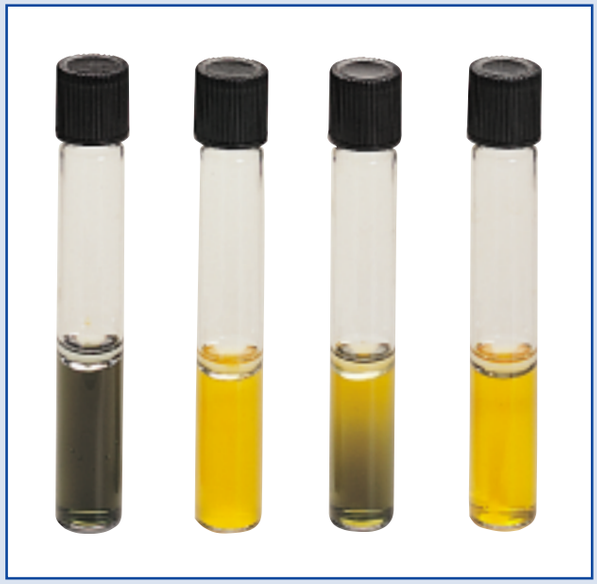
Prepare OF Basal Medium per label directions. Inoculate duplicate tubes, overlay one tube with 2 ml sterile mineral oil, and incubate at 35°C for 40-48 hours.

ORGANISM	ATCC®	PLAIN OPEN	PLAIN CLOSED	w/1% DEXTROSE OPEN	w/1% DEXTROSE CLOSED
Acinetobacter calcoaceticus	19606	K	K	A	K
Escherichia coli	25922*	K	K	AG	AG
Pseudomonas aeruginosa	27853*	K	K	A	K
Shigella flexneri	12022*	K	K	A	A

K = Alkaline reaction, green to blue medium
A = Acid reaction, yellow medium
G = Gas

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

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



Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Acinetobacter calcoaceticus* ATCC® 19606 | *Shigella flexneri* ATCC® 12022

Tubes above are closed, with Dextrose

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

OF Basal Medium

Materials Required But Not Provided

Glassware
Autoclave
Sterile melted petrolatum (mineral oil)
Carbohydrates of choice: Glucose (dextrose), Lactose, Maltose, Mannitol, Saccharose and/or Xylose
Incubator (35°C)
Test tubes with closures

Method of Preparation

1. Suspend 9.4 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Add 1% carbohydrate before or after sterilization, depending on heat lability.
5. Dispense in test tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate duplicate test tubes by stabbing the medium to the desired depth with an inoculating needle.
2. Overlay one tube of each set with 2 ml sterile mineral oil.
3. Incubate at a temperature appropriate for the test organism for a minimum of 48 hours.
4. Examine the medium for gas production and a color change from green to yellow.

Results

Alkaline reaction: green to blue medium
Acid reaction: yellow medium

A color change of the medium in both the plain and overlay tubes, with or without gas production, indicates fermentation of the carbohydrate tested.

A color change in the plain tube, only, indicates oxidative metabolism of the carbohydrate tested.

No color change in both tubes of the set indicates the organism is nonsaccharolytic for the carbohydrate tested.

Limitations of the Procedure

1. The acid reaction produced by oxidative organisms is apparent at the surface and gradually spreads throughout the medium. If the oxidation is weak or slow, however, an initial alkaline reaction at the surface of the open tube may persist for several days and eventually convert to an acid reaction.
2. If an organism is unable to grow on OF Basal Medium, Cowan[10] recommends adding either 2% serum or 0.1% yeast extract to each carbohydrate tube.
3. Nonsaccharolytic organisms produce a slight alkalinity in the open tube (blue-green color), while the covered tube will not exhibit a color change (green).

References

1. **Hugh, R. and E. Leifson.** 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. **66**:24-26.
2. **Bacteriological Analytical Manual, 8th ed.** 1995. AOAC International. Gaithersburg, MD.
3. **Vanderzant, C. and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1992. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Oberhofer, T. R.** 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, NY.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
7. **Knapp, J. S. and K. K. Holmes.** 1983. Modified oxidation-fermentation medium for detection of acid production from carbohydrates by *Neisseria* spp. and *Branhamella catarrhalis*. J. Clin. Microbiol. **18**:56-62.
8. **Welch, D. F., M. J. Muszynski, C. H. Pai, M. J. Marcon, M. M. Hribar, P. H. Gilligan, J. M. Matsen, P. A. Ahlin, B. C. Hilman and S. A. Chartrand.** 1987. Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. J. Clin. Microbiol. **25**:1730-1734.
9. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
10. **Cowan, S. T.** 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge, MA.

Packaging

OF Basal Medium	100 g	0688-15
	500 g	0688-17
Mineral Oil	30 ml	6663-30

OGYE Agar

Bacto® OGYE Agar Base · Bacto Antimicrobic Vial Oxytetracycline

Intended Use

Bacto OGYE Agar Base is for use with Bacto Antimicrobic Vial Oxytetracycline in isolating and enumerating yeasts and molds in foods.

Also Known As

OGY Agar Base[1]

OGYA (Oxytetracycline-Glucose-Yeast (Extract) Agar)[1]

Summary and Explanation

Acidified agar may be used for enumerating yeasts and molds in foods and dairy products. However, in some cases, antimicrobics better suppress bacterial growth and improve recovery of yeasts and molds.[2, 3]

Mossel et al.[4, 5] described Oxytetracycline-Glucose Yeast Extract (OGYE) Agar for selectively isolating and enumerating yeasts and molds in foods. Mossel et al. demonstrated improved recovery compared to acidified agar media.

User Quality Control

Identity Specifications

OGYE Agar Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, very slightly opalescent without precipitate.
Prepared Medium:	Medium amber, slightly opalescent.
Reaction of 3.7% Solution at 25°C:	pH 7.0 ± 0.2

Antimicrobic Vial Oxytetracycline

Lyophilized Appearance: Yellow cake or powder

Cultural Response

Prepare OGYE Agar Base and Antimicrobic Vial Oxytetracycline per label directions. Inoculate using pour plate technique and incubate at 22 ± 3°C for up to 5 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Escherichia coli	25922*	1,000-2,000	inhibited
Saccharomyces cerevisiae	9763	100-1,000	good
Saccharomyces carlsbergensis† (cerevisiae or uvarum)	9080	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.

†*S. cerevisiae* ATCC 9080 is prepared to order.

OGYE Agar is specified as a standard methods medium for use with dairy products.[2]

Principles of the Procedure

OGYE Agar Base contains Yeast Extract to supply B-complex vitamins which stimulate bacterial growth. Dextrose is the carbon energy source. Bacto Agar is the solidifying agent. Antimicrobic Vial Oxytetracycline inhibits the growth of bacteria.

Formula

OGYE Agar Base

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Dextrose	20 g
Bacto Agar	12 g

Final pH 7.0 ± 0.2 at 25°C

Antimicrobic Vial Oxytetracycline

Oxytetracycline	100 mg

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 antimicrobic supplement 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

OGYE Agar Base
Antimicrobic Vial Oxytetracycline

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave

Method of Preparation

1. Suspend 37 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 50°C.

4. Aseptically add 10 ml rehydrated Antimicrobic Vial Oxytetracycline to the medium. Mix well.

Antimicrobic Vial Oxytetracycline

1. Aseptically add 10 ml sterile distilled or deionized to the Antimicrobic Vial Oxytetracycline.
2. Shake to dissolve contents.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 579-582. Williams & Wilkins, Baltimore, MD.
2. **Frank, J. F., G. L. Christen, and L. B. Bullerman**. 1993. Tests for groups of microorganisms, p. 271-286. *In* R. T. Marshall (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Mislivec, P. B., L. R. Beuchat, and M. A. Cousin.** 1992. Yeasts and molds, p. 239-249. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Mossel, D. A. A., A. M. C. Kleynen-Semmeling, H. M. Vincentie, H. Beerens, and M. Catsaras**. 1970. Oxytetracycline-Glucose-Yeast Extract Agar for selective enumeration of moulds and yeasts in foods and clinical material. J. Appl. Bacteriol. **33**:454-457.
5. **Mossel, D. A. A., M. Visser, and W. H. J. Mengerink**. 1962. A comparison of media for the enumeration of moulds and yeasts in food and beverages. Lab. Pract. **11**:109-112.

Packaging

OGYE Agar Base	500 g	1811-17
Antimicrobic Vial Oxytetracycline	10 ml	3267-59

Bacto® Oatmeal Agar

Intended Use

Bacto Oatmeal Agar is used for cultivating fungi, particularly for macrospore formation.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, nonhomogeneous, may be slightly lumpy.
Solution:	7.25% solution, soluble in distilled or deionized water on boiling with frequent agitation. Solution is off-white, opaque with nonhomogeneous particles.
Prepared Medium:	Off-white, opaque appearance with nonhomogeneous particles.
Reaction of 7.25% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Prepare Oatmeal Agar per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

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

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

Summary and Explanation

Fungi are extremely successful organisms, as evidenced by their ubiquity in nature.[1] Of the estimated 250,000 species, fewer than 150 are known primary pathogens of humans.[1]

Identification and classification of fungi is primarily based on the morphologic differences in their reproductive structures.[2] Fungi reproduce by producing spores.[2] Large, multi-celled spores are called macroconidia, macroaleuriospores or macrospores and are produced by aerial sporulation.[2]

The detection of fungi is a great concern in the pharmaceutical, food and cosmetic industry.

Principles of the Procedure

Oatmeal is a source of nitrogen, carbon, protein and nutrients. Bacto Agar is a solidifying agent.

Formula

Oatmeal Agar

Formula Per Liter

Oatmeal	60 g
Bacto Agar	12.5 g

Final pH 6.0 ± 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.

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

Oatmeal Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Sterile Petri dishes
Waterbath (optional)

Method of Preparation

1. Suspend 72.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling with constant agitation.
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

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

Results

Refer to appropriate references and procedures for results.

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. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **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

Oatmeal Agar	500 g	0552-17

Bacto® Orange Serum Agar
Bacto Orange Serum Broth Concentrate 10X

Intended Use

Bacto Orange Serum Agar is used for cultivating aciduric microorganisms, particularly those associated with spoilage of citrus products.

Bacto Orange Serum Broth Concentrate 10X is used for cultivating and enumerating microorganisms associated with spoilage of citrus products.

Summary and Explanation

The low pH of fruit juices makes citrus fruit products susceptible to spoilage by yeasts, molds and the bacteria *Lactobacillus* and *Leuconostoc*.[1] In the 1950's, Hays investigated spoilage in frozen concentrated orange juice. He found that an agar medium containing orange serum (juice) was superior to Lindegren Agar in isolating the microorganisms responsible for spoilage causing a buttermilk off-odor.[2] In a later comparative study, Murdock, Folinazzo and Troy found Orange Serum Agar, pH 5.4 to be a suitable medium for growing *Leuconostoc*, *Lactobacillus* and yeasts.[3]

Orange Serum Agar is included in recommended methods for examining fruit beverages.[1] Orange Serum Broth Concentrate 10X is used for small samples to initiate growth of saprophytic and pathogenic fungi.[4]

Principles of the Procedure

Orange Serum Agar and Orange Serum Broth Concentrate 10X contain Tryptone as carbon and nitrogen sources for general growth requirements. Orange serum provides the acid environment favorable to recovering acid-tolerant microorganisms. Yeast Extract supplies B-complex vitamins which stimulate growth. Dextrose is the carbohydrate. Bacto Agar is the solidifying agent (Orange Serum Agar only).

Formula

Orange Serum Agar

Formula Per Liter

Orange Serum	200 ml
Bacto Yeast Extract	3 g
Bacto Tryptone	10 g
Bacto Dextrose	4 g
Dipotassium Phosphate	2.5 g
Bacto Agar	17 g

Final pH 5.5 ± 0.2 at 25°C

Orange Serum Broth Concentrate 10X

Formula Per Liter

Orange Serum Concentrate	100 ml
Bacto Yeast Extract	3 g
Bacto Tryptone	10 g
Bacto Dextrose	4 g
Dipotassium Phosphate	2.5 g

Final pH 5.6 ± 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 Orange Serum Agar and the Orange Serum Broth Concentrate 10X at 2-8°C.

The dehydrated Orange Serum Agar 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

Orange Serum Agar
Orange Serum Broth Concentrate 10X

Materials Required but not Provided

For Orange Serum Agar:

Flask with closure
Distilled or deionized water
Autoclave

For Orange Serum Broth Concentrate 10X:

Sterile distilled or deionized water
Sterile test tubes
Sterile transfer pipettes, 10 ml

Method of Preparation

Orange Serum Agar

1. Suspend 45.5 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 which could cause a softer medium.

Orange Serum Broth Concentrate 10X

1. To prepare the single-strength medium, aseptically add 100 ml Orange Serum Concentrate 10X to 900 ml sterile distilled or deionized water and mix thoroughly.
2. Use aseptic technique to dispense 10 ml amounts into sterile test tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Orange Serum Agar

1. For plate count method, prepare serial 10-fold dilutions of the test material.
2. Add 1 ml of test sample to sterile Petri dish.
3. Add 18-20 ml of sterile, molten agar (cooled to 45-50°C) and swirl plate gently to mix well.
4. Allow to solidify before incubating at 30°C for 48 hours. Plates can be held up to 5 days.

Orange Serum Broth Concentrate 10X

Orange Serum Broth Concentrate 10X is used for small samples to initiate growth.

Results

Orange Serum Agar

Record colony morphology for each type of growth.

Orange Serum Broth Concentrate 10X

Turbidity indicates growth.

User Quality Control

Identity Specifications

Orange Serum Agar

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
4.55% Solution:	Light to medium amber, very slightly to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 4.55% Solution at 25°C:	pH 5.5 ± 0.2

Orange Serum Broth Concentrate 10X

Concentrate Appearance:	Dark amber, clear solution.
Reaction of Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Prepare media per label directions. Inoculate medium and incubate for 40-48 hours. *Lactobacillus* is incubated at 35 ± 2°C and the remaining organisms are incubated at 30 ± 2°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Lactobacillus fermentum	9338	100-1,000	good
Leuconostoc mesenteroides subsp. *mesenteroides*	23386	100-1,000	good
Sacharomyes cerevisiae	9763	100-1,000	good

Limitations of the Procedure

1. Orange Serum Agar is not a differential medium. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.
2. If Orange Serum Agar is divided into aliquots and allowed to solidify, remelt only once. Repeated heating may produce a softer medium.

References

1. **Vanderzant, C., and D.F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Hays, G. L.** 1951. The isolation, cultivation and identification of organisms which have caused spoilage in frozen concentrated orange juice. Proc. Fla. State Hortic. Soc. **54**:135-137.
3. **Murdock, D. I., J. F. Folinazzo, and V. S. Troy**. 1952. Evaluation of plating media for citrus concentrates. Food Technol. **6**:181-185.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Orange Serum Agar	500 g	0521-17*
Orange Serum Broth Concentrate 10X	6 x 100 ml	0518-73*

*Store at 2-8°C

Bacto® Oxford Medium Base · Bacto Oxford Antimicrobic Supplement · Bacto Modified Oxford Antimicrobic Supplement

Intended Use

Bacto Oxford Medium Base is used with either Bacto Oxford Antimicrobic Supplement[1] or Bacto Modified Oxford Antimicrobic Supplement[2] for isolating and differentiating *Listeria monocytogenes*.

Summary and Explanation

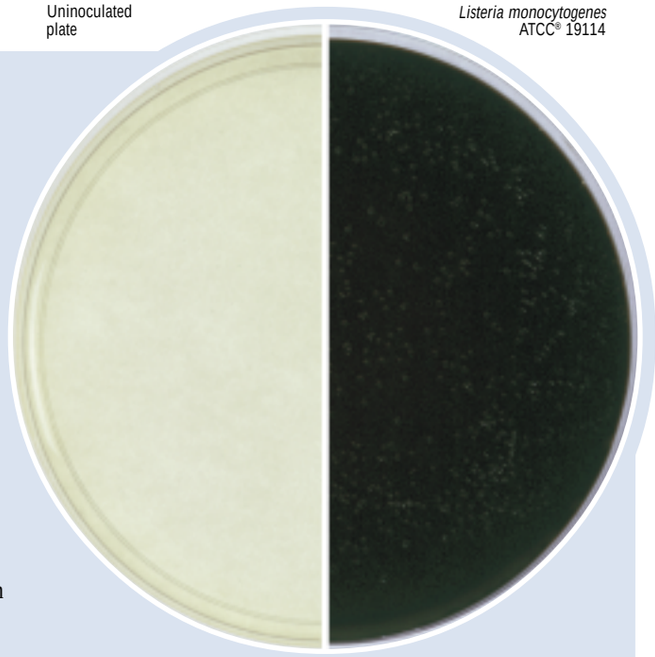
First described in 1926 by Murray, Webb and Swann,[3] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[4] The first reported food-borne outbreak of listeriosis was in 1985.[5] Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[6]

Implicated vehicles of transmission include turkey frankfurters,[7] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[8]

Listeria spp. grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside these parameters.[9] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[10]

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



User Quality Control

Identity Specifications

Oxford Medium Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.75% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, slightly opalescent with a blue ring at the surface of the liquid.
Prepared Medium:	Light to medium amber, very slightly to slightly opalescent.
Reaction of 5.75% Solution at 25°C:	pH 7.2 ± 0.2

Oxford Antimicrobic Supplement

Appearance:	Yellow cake; yellow solution upon rehydration.

Modified Oxford Antimicrobic Supplement

Appearance:	White to off-white cake; colorless solution upon rehydration.

Cultural Response

Prepare Oxford Medium or Modified Oxford Medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY ON OXFORD MEDIUM	RECOVERY ON MODIFIED OXFORD MEDIUM
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition	marked to complete inhibition
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	marked to complete inhibition
Listeria monocytogenes	19114	100-1,000	good at 40-48 hours, black colonies	good at 40-48 hours, black colonies
Saccharomyces pastorianus	9080	1,000-2,000	marked to complete inhibition	(not tested)

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 Oxford Medium Base is prepared according to the formulation of Curtis et al.[11] who originally described the medium and its use in the selective isolation of *Listeria* from mixed cultures.

Principles of the Procedure

Columbia Blood Agar Base combines Pantone, Bitone and Tryptic Digest of Beef Heart which provide nitrogen, carbon, amino acids and vitamins. Bacto Agar is the solidifying agent. Sodium Chloride maintains the osmotic balance.

Ferric ammonium citrate aids in the differentiation of *Listeria* spp. Since all *Listeria* spp. hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the colony and surrounding medium in cultures containing esculin-hydrolyzing bacteria results from the formation of 6,7-dihydroxycoumarin which reacts with the ferric ions.[12]

Selectivity is provided by the presence of Lithium Chloride in the formula. The high salt tolerance of *Listeria* is used as a means to markedly inhibit growth of enterococci.

Selectivity is increased by adding various antimicrobial agents to the base. Incorporating these agents into Oxford Medium Base will completely inhibit gram-negative organisms and most gram-positive organisms after 24 hours of incubation. The most widely recognized antimicrobial agent combinations are the Oxford Medium formulation[11] and the Modified Oxford Medium formulation.[2] The Oxford Medium formulation contains cycloheximide, colistin sulfate, acriflavin, cefotetan and fosfomycin (available as Oxford Antimicrobic Supplement). The Modified Oxford Medium formulation contains moxalactam and colistin methane sulfonate or colistin sulfate (available as Modified Oxford Antimicrobic Supplement).

Modified Oxford medium is recommended for isolating and identifying *Listeria monocytogenes* from processed meat and poultry products.[2] Oxford Medium is recommended for isolating *Listeria* from enrichment broth cultures.[13]

Formula

Oxford Medium Base

Formula Per Liter

Bacto Columbia Blood Agar Base	39 g
Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Lithium Chloride	15 g
Bacto Agar	2 g

Final pH 7.2 ± 0.2 at 25°C

Oxford Antimicrobic Supplement

Ingredients per 10 ml vial

Acriflavine	5 mg
Cefotetan	2 mg
Colistin Sulfate	20 mg
Cycloheximide	400 mg
Fosfomycin	10 mg

Modified Oxford Antimicrobic Supplement

Ingredients per 10 ml vial

Colistin Sulfate	10 mg
Moxalactam	20 mg

Precautions

1. For Laboratory Use.
2. **Oxford Medium 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 Organs: Blood, Kidneys, Nerves.

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

   Oxford Antimicrobic Supplement

   Warning! HARMFUL IF SWALLOWED OR INHALED. MAY CAUSE IRRITATION. MAY CAUSE BIRTH DEFECTS. Avoid contact with eyes, skin and clothing. Do not breathe dust.

   FIRST AID: If swallowed, induce vomiting. Call a physician. Never give anything by mouth to an unconscious person. If inhaled, remove to fresh air. If not breathing, give artificial respiration, preferably mouth-to-mouth. If breathing is difficult, give oxygen. Call a physician. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician. Flush skin with water.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

1. Store Oxford Medium Base below 30°C dehydrated powder is very hygroscopic. Keep container tightly closed.

   Store Oxford Antimicrobic Supplement and Modified Oxford Antimicrobic Supplement at 2-8°C.
2. Store prepared media at 2-8°C.

Expiration Date

The expiration date applies to the product 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

Oxford Medium Base
Oxford Antimicrobic Supplement
Modified Oxford Antimicrobic 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 57.5 grams of Oxford Medium Base in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Cool medium to 45-50°C in a waterbath.
5. **To prepare Oxford Medium:** Aseptically rehydrate one vial of Oxford Antimicrobic Supplement with 5 ml ethanol and 5 ml sterile distilled water. Rotate in an end-over-end motion to dissolve completely. Add 10 ml to 1 liter sterile Oxford Medium Base.

   To prepare Modified Oxford Medium: Aseptically rehydrate one vial of Modified Oxford Antimicrobic Supplement with 10 ml sterile distilled water. Rotate in an end-over-end motion to dissolve completely. Add 10 ml to 1 liter sterile Oxford Medium Base.

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.[1,2,9,13,14]
2. Clinical specimens obtained from nonsterile sites, foods and specimens obtained from the environment should be selectively enriched for *Listeria* before being plated.[14]
3. Process each specimen using procedures appropriate for that specimen or sample.[1,2,9,13,14]

Test Procedure

The USDA method[2] involves enrichment of the food sample in UVM Modified Listeria Enrichment Broth (one part sample to nine parts broth) at 30°C. After incubation, a portion of the enrichment mixture is plated onto Oxford or Modified Oxford Medium.

The FDA method[1] involves adding 25 ml of liquid or 25 grams of solid material to 225 ml Listeria Enrichment Broth and incubating at 30°C for two days. After enrichment, the broth is plated onto Oxford Medium.

For further information when testing food samples or clinical specimens for *Listeria*, consult appropriate references.[1,2,9,13,14]

Results

Select esculin-positive colonies and confirm their identity by further biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification. For additional information, refer to appropriate references.[1,2,9,13,14]

Limitations of the Procedure

1. Since *Listeria* spp. other than *L. monocytogenes* can grow on these media, an identification of *L. monocytogenes* must be confirmed by biochemical and serological testing.[14]
2. Use freshly prepared antimicrobial agent solutions or aliquot portions and store at -20°C or below.
3. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

References

1. **Hitchins, A. D.** 1992. *Listeria monocytogenes*. Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
2. **Lee, W. H., and D. McClain.** 1989. *Laboratory Communication No. 57* (revised May 24, 1989). U.S.D.A., F.S.I.S. Microbiology Division, Beltsville, MD.
3. **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.
4. **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.
5. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma special report. J. Assoc. Off. Anal. Chem. **70**:769-772.
6. **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.
7. **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.
8. **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.
9. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*, p. 637-663. *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.
10. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
11. **Curtis, G. D. W., R. G. Mitchell, A. F. King, and J. Emma.** 1989. A selective differential medium for the isolation of *Listeria monocytogenes*. Appl. Microbiol. **8:**95-98.
12. **Fraser, J., and W. Sperber.** 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. **51:**762-765.
13. **Chesemore, R. G.** 1990. Bacteriological analytical manual, Chapter 29 - *Listeria* isolation: culture medium substitution in method of analysis. Federal Register **55(183):**38753-4.
14. **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, R. H. Yolken (ed), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Oxford Medium Base	500 g	0225-17
	2 kg	0225-07
	10 kg	0225-08
Oxford Antimicrobic Supplement	6 x 10 ml	0214-60
Modified Oxford Antimicrobic Supplement	6 x 10 ml	0218-60