Agar

Bacto® Agar · Agar Flake · Agar, Granulated · Agar Noble Agar Bacteriological Technical

Intended Use

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

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

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

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

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

Summary and Explanation

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

Agar was first suggested for microbiological purposes in 1881 by Fannie Hesse.[1, 2] By the early 1900s, agar became the gelling agent of choice over gelatin because agar remains firm at growth temperatures

User Quality Control

Identity Specifications

	BACTO® AGAR	AGAR FLAKE	AGAR, GRANULATED	AGAR NOBLE	AGAR BACTERIOLOGICAL TECHNICAL
Dehydrated Appearance:	Very light beige, free flowing, homogeneous, granules.	Off-white to light beige, free flowing, flakes.	Very light beige to light tan, free flowing homogeneous, granules.	White to off-white, free flowing,homogeneous, fine granules.	Very light to medium beige, free flowing, homogeneous.
Solution 1.5% solution soluble in distilled or deionized water upon boiling	Solution is very light amber; very slightly to slightly opalescent. Clarity is less than 10 Nephelometric turbidity units.	Solution is very light to light amber, very slightly to slightly opalescent.	Solution is very light to medium amber, very slightly opalescent to opalescent.	Solution is colorless, clear to very slightly opalescent.	Solution is very light to medium amber, opalescent.
Loss on Drying (LOD)	16-20%	Less than or equal to 20%	Less than or equal to 20%	Less than or equal to 20%	Less than or equal to 20%
Ash[6]	Less than or equal to 6.5%	2-5.2%	Less than or equal to 6.5%	Less than or equal to 2%	Less than or equal to 6.5%
Calcium µg/g (ppm)	300-3,000 ppm	Less than or equal to 3,400 ppm	Less than or equal to 3,000 ppm	100-2,600 ppm	Less than or equal to 3,000 ppm
Magnesium µg/g (ppm)	50-1,000 ppm	Less than or equal to 1,850 ppm	Less than or equal to 1,000 ppm	0-750 ppm	Less than or equal to 1,300 ppm
Melting Point	83-89°C	Greater than or equal to 85°C	83-89°C	Greater than or equal to 85°C	Greater than or equal to 85°C
Gelation Point	32-39°C	32-39°C	32-39°C	32-39°C	32-39°C

Cultural Response

Prepare the agar formulation of Nutrient Broth (0003) or LB Broth, Miller (0446) by adding 1.5% agar. Sterilize and pour plates. Inoculate with 100-1,000 CFU of the indicated test organisms and incubate at 35 ± 2°C for 18-24 hours. Record recovery.

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

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

Can of Bacto Agar

for many pathogens. Agar is also generally resistant to a breakdown by bacterial enzymes. The use of agar in microbiological media significantly contributed to the advance of microbiology, paving the way for pure culture isolation and study.

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

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

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

Product Applications

Bacto® Agar is optimized for beneficial calcium and magnesium content. Detrimental ions such as iron and copper are reduced. Bacto® Agar is recommended for clinical applications, auxotrophic studies, bacterial and yeast transformation studies, and bacterial molecular genetics applications.[4, 5]

Agar Flake is recommended for general bacteriological purposes. The quality is similar to Bacto® Agar. However, the flakes are more easily wetted than the granules found in Bacto® Agar.

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

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

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

Typical Analysis

	BACTO® AGAR	AGAR, GRANULATED	AGAR NOBLE	AGAR BACTERIOLOGICAL TECHNICAL
Physical Characteristics				
Ash (%)	3.6	3.4	1.3	4.1
Color	lt. beige	lt. beige	off white	lt beige
Texture	granular free-flowing	granular free-flowing	fine granular free flowing	granular free-flowing
Clarity, 1.5% Soln (NTU)	4.3	5.3	3.7	26.2
Loss on Drying (%)	17.3	12.2	16.0	18.2
pH, 1.5% Soln	6.5	6.6	5.7	6.9
Gel Strength (g/cm^2)	600	560	700	613
Gelation Point(°C)	35°C	35°C	35°C	36°C
Melting Point (°C)	88°C	88°C	87°C	88°C
Biological Testing (CFU/g)				
Spore Count	<1,000	<1,000	<1,000	4,300
Standard Plate Count	<1,000	<1,000	<1,000	2,725
Inorganics (%)				
Calcium	0.179	0.133	0.015	0.110
Chloride	0.021	<0.005	<0.050	0.172
Cobalt	<0.001	<0.001	<0.001	<0.001
Copper	<0.001	<0.001	<0.001	<0.001
Iron	0.002	0.003	<0.001	0.002
Lead	<0.001	<0.001	<0.001	<0.001
Magnesium	0.068	0.041	0.002	0.093
Manganese	<0.001	<0.001	<0.001	<0.001
Nitrate	<0.005	<0.005	<0.050	<0.005
Phosphate	<0.005	0.010	<0.050	0.015
Potassium	0.121	0.079	0.022	0.124
Sodium	0.837	0.776	0.335	0.932
Sulfate	1.778	1.710	0.663	0.367
Sulfur	0.841	0.868	0.333	0.646
Tin	<0.001	<0.001	<0.001	<0.001
Zinc	<0.001	<0.001	<0.001	<0.001

Precautions

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

Storage

Store dehydrated agar below 30°C. Dehydrated 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 the product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto® Agar
Agar Flake
Agar, Granulated
Agar Noble
Agar Bacteriological Technical

Materials Required But Not Provided

Materials vary depending on the application.

Method of Preparation

Method of preparation varies depending on the application.

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 Bacto® Agar, Agar Flake, Agar, Granulated, Agar Noble or Agar Bacteriological Technical.

Results

Refer to appropriate references and procedures for results.

References

1. **Hesse, W.** 1894. Über die quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen. Mitt. a.d. Kaiserl. Gesh. Berlin **2**:182-207.
2. **Hitchens, A. P., and M. C. Leikind.** 1939. The introduction of agar-agar into bacteriology. J. Bacteriology **37**:485-493.
3. **Selby, H. H., and T. A. Selby.** 1959. Agar. *In* Whister (ed.), Industrial gums. Academic Press Inc., New York, NY.
4. **Sambrook, J., E. F. Fritsch, and T. Maniatis**. 1989. Molecular cloning, a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, NY, NY.
5. **Schiestl, R. H., and R. Daniel Geitz**. 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Current Genetics **16**:339-346.
6. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.

Packaging

Bacto® Agar	100 g	0140-15
	1 lb	0140-01
	2 kg	0140-07
	10 kg	0140-08
Agar Flake	500 g	0970-17
Agar, Granulated	100 g	0145-17
	2 kg	0145-07
	10 kg	0145-08
Agar Noble	100 g	0142-15
	500 g	0142-17
Agar Bacteriological Technical	500 g	0812-17
	2 kg	0812-07
	10 kg	0812-08

Bacto® 2xYT

Intended Use

Bacto 2xYT is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

2xYT is a nutritionally rich growth medium designed for growth of recombinant strains of *Escherichia coli*. This medium is also used for propagation of M13 bacteriophage for sequencing and phage display research.[1-3] The components of 2xYT provide nitrogen and growth factors that allow bacteriophage to reproduce in large quantities without exhausting the host. *E. coli* grows more rapidly in this rich medium because it provides amino acids, nucleotide precursors, vitamins and other metabolites that the cell would otherwise have to synthesize.[2]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 3.1% Solution 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare 2xYT 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
Escherichia coli (JM103)	39403	100-300	Good
Escherichia coli (JM107)	47014	100-300	Good
Escherichia coli (HB101)	33694	100-300	Good
Escherichia coli (DH-1)	33849	100-300	Good
Escherichia coli (DH-5)	53868	100-300	Good

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

Principles of the Procedure

Tryptone and Yeast Extract provide the necessary nutrients and cofactors required for excellent growth of *E. coli*. Sodium Chloride is included to provide a suitable osmotic environment.

Formula

2xYT

Formula Per Liter	
Bacto Tryptone	16 g
Bacto Yeast Extract	10 g
Sodium Chloride	5 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

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

Storage

Store the dehydrated medium below 30°C. The 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

2xYT

Materials Required But Not Provided

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

Method of Preparation

1. Dissolve 31 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Please consult appropriate references for recommended test procedures.[1-3]

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.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl**. 1994. Current protocols in molecular biology, vol 1. Current Protocols, New York, N.Y.
3. **Davis, L. G., M. D. Dibner, and J. F. Battey**. 1986. Basic methods in molecular biology. Elsevier, New York, N.Y.

Packaging

2xYT	500 g	0440-17-0

Bacto® A-1 Medium

Intended Use

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

Also Known As

A-1 Medium is also referred to as A-1 Broth.

Summary and Explanation

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

User Quality Control

Identity Specifications

Dehydrated Appearance: Light beige, lumpy.

Solution: 3.15% solution, soluble in distilled or deionized water on boiling. Solution is light amber, opalescent immediately after sterilization. Solution is light amber, clear, may have flocculent precipitate upon cooling.

Prepared Medium: (When cooled to room temperature) - Light amber, clear, flocculent precipitate may be present.

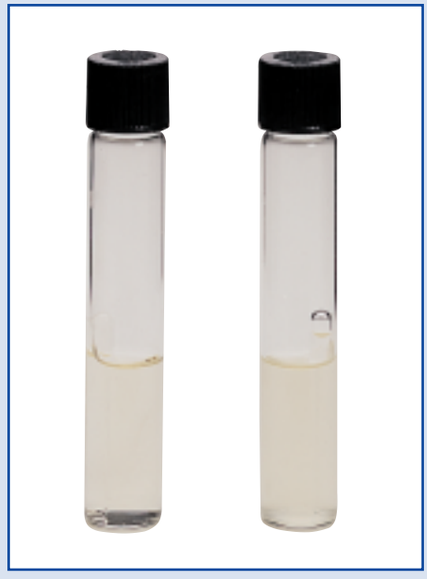
Reaction of 3.15% Solution at 25°C: pH 6.9 ± 0.1

Cultural Response

Prepare A-1 Medium per label directions. Prepare tubes by placing fermentation vials and 10 ml amounts of medium into tubes. Inoculate and incubate at 35 ± 2°C for 3 hours. Transfer tubes to a 44.5°C waterbath for 21 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU(APPROX.)	GROWTH
Bacillus subtilis†	6633	100	none
Enterobacter aerogenes	13048*	100	poor to good/may produce gas
Enterococcus faecalis	19433*	100	none to poor
Escherichia coli	25922*	100	good/with gas production
Escherichia coli	13762	100	good/with gas production

The cultures listed are the minimum that should be used for performance testing.
†*Bacillus subtilis* is available as Subtilis Spore Suspension.



Uninoculated tube

Escherichia coli ATCC® 25922 with fermentation vial

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

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

A-1 Medium can be used in a single-step procedure for the detection of fecal coliforms in source water, seawater, treated wastewater and foods. Prior enrichment in a presumptive medium is not required.[4] A-1 Medium conforms to standard methods for the isolation of fecal coliforms in water and foods.[4,5,6]

Principles of the Procedure

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

Formula

A-1 Medium

Formula Per Liter	
Bacto Tryptone	20 g
Bacto Lactose	5 g
Sodium Chloride	5 g
Bacto Salicin	0.5 g
Triton X-100	1 ml

Final pH 6.9 ± 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 the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared medium in the dark at room temperature for no longer than 7 days.[4]

Expiration Date

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

Procedure

Materials Provided

A-1 Medium

Materials Required But Not Provided

Glassware
Fermentation vials
Autoclave
Incubator (35°C)
Waterbath (44.5°C)
Test tubes
Distilled or deionized water

Method of Preparation

1. Suspend 31.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 10 minutes.

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

Specimen Collection and Preparation

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

Test Procedure

1. Inoculate tubes of A-1 Medium as directed in standard methods.[4,5,6]
2. Incubate at 35 ± 0.5°C for 3 hours.
3. Transfer tubes to a water bath at 44.5 ± 0.2°C and incubate for an additional 21 ± 2 hours.
4. Maintain water level in bath above level of liquid in inoculated tubes.

Results[5]

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

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Fecal coliform counts are usually greater than *E. coli* counts.[5]
3. Interpretation of test procedure using A-1 Medium requires understanding of the microflora of the specimen.[5]

References

1. **Andrews, W. H., C. D. Diggs, and C. R. Wilson.** 1975. Evaluation of a medium for the rapid recovery of *Escherichia coli* from shellfish. Appl. Microbiol. **29**:130- 131.
2. **Andrews, W. H., and M. W. Presnell.** 1972. Rapid recovery of *Escherichia coli* from estuarine water. Appl. Microbiol. **23**:521-523.
3. **Standridge, and Delfino.** 1981. Appl. Environ. Microbiol. **42**:918.
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. **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.

Packaging

A-1 Medium	500 g	1823-17

Bacto® AC Broth
Bacto AC Broth w/o Dextrose

Intended Use

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

Bacto AC Broth w/o Dextrose is used, with the addition of a carbohydrate, for cultivating a wide variety of microorganisms.

Summary and Explanation

AC Broth and AC Broth w/o Dextrose possess growth-promoting properties for voluminous growth of a wide variety of microorganisms. Christensen[1] and Malin and Finn[2] reported that AC Medium does not exhibit the toxicity shown by media containing sodium thioglycollate.

User Quality Control

Identity Specifications

AC Broth

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

AC Broth w/o Dextrose

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.92% solution, soluble in distilled or deionized water. Solution is medium to dark amber, clear to very slightly opalescent.
Prepared Tubes:	Medium to dark amber, clear to very slightly opalescent.
Reaction of 2.92% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare AC Broth or AC Broth w/o Dextrose per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Corynebacterium diphtheriae Type *mitis*	8024	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

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

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

Several early studies reported on the wide variety of organisms able to grow on AC Medium.[3,4,5] AC Broth is suitable for use in the detection of obligately aerobic contaminants in biologicals and other products. AC Broth and AC Broth w/o Dextrose are also useful in the isolation and cultivation of many common pathogenic and saprophytic aerobes.[6] The media can be used to test the sterility of biologicals and solutions that do not contain mercurial preservatives. Fluid Thioglycollate Medium should be employed for the sterility testing of solutions containing mercurial preservatives.

AC Broth w/o Dextrose has the same formula as AC Broth except that the dextrose is omitted, allowing for the addition of other carbohydrates if desired.

Principles of the Procedure

Proteose Peptone No. 3, Beef Extract, and Malt Extract provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon are provided by Yeast Extract. Dextrose is included in AC Broth as a carbon energy source. Ascorbic Acid is added to clarify the solution.

Formula

AC Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Bacto Beef Extract	3 g
Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Bacto Dextrose	5 g
Ascorbic Acid	0.2 g

Final pH 7.2 ± 0.2 at 25°C

AC Broth w/o Dextrose

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Bacto Beef Extract	3 g
Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Ascorbic Acid	0.2 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 media below 30°C. The dehydrated media are very hygroscopic. Keep container tightly closed.

AC Broth

Store prepared medium at 15-30°C. After prolonged storage, reheat in flowing steam or a boiling water bath for a few minutes to drive off dissolved gases. Cool without agitation.

AC Broth w/o Dextrose

Store prepared medium at 15-30°C.

Expiration Date

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

Procedure

Materials Provided

AC Broth
AC Broth w/o Dextrose

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend appropriate amount of medium in 1 liter distilled or deionized water:
   AC Broth - 34 grams;
   AC Broth w/o Dextrose - 29.2 grams.
2. If necessary, warm slightly to dissolve completely.
3. Dispense as desired. Autoclave at 121° C for 15 minutes.
   If the medium is not used the same day it is sterilized, place in flowing steam or a boiling water bath for a few minutes to drive off dissolved gases. Allow to cool without agitation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

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

References

1. Paper read at New York Meeting. Am. Pub. Health Assoc., 1944.
2. **Malin, B., and R. K. Finn.** 1951. The use of a synthetic resin in anaerobic media. J. Bacteriol. **62**:349-350.
3. **Reed, G. B., and J. H. Orr.** 1943. Cultivation of anaerobes and oxidative-reduction potentials. J. Bacteriol. **45**:309-320.
4. **Schneiter, R., J. E. Dunn, and B. H. Caminita.** 1945. Studies in connection with the selection of a satisfactory culture medium for bacterial air sampling. Pub. Health Reports **60**:789-806.
5. **Kolb, R. W., and R. Schneiter.** 1950. The germicidal and sporicidal efficacy of methyl bromide for *Bacillus anthracis*. J. Bacteriol. **59**:401-412.
6. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 13-14. Williams & Wilkins, Baltimore, MD.

Packaging

AC Broth	500 g	0317-17
AC Broth w/o Dextrose	10 kg	0599-08

Bacto® APT Agar
Bacto APT Broth

Intended Use

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

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

Also Known As

All Purpose Tween

Summary and Explanation

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

The lactic acid bacteria, a group of acid producing bacteria, include the genera *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Lactobacillus*.[3] These organisms are widespread in nature and are associated with bacterial spoilage of foods such as dairy, meat and vegetable products.[3] One use of APT Agar and APT Broth is for cultivating these heterofermentative lactic acid bacteria from food products.[3]

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

Principles of the Procedure

APT Agar and APT Broth contain Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate. The Manganese Chloride, Magnesium Sulfate and Ferrous Sulfate provide ions used in replication by lactobacilli. Sorbitan Monooleate Complex is a source of fatty acids required by lactobacilli. Bacto Agar is the solidifying agent in APT Agar.

Formula

APT Agar

Formula Per Liter

Bacto Yeast Extract	7.5 g
Bacto Tryptone	12.5 g
Bacto Dextrose	10 g
Sodium Citrate	5 g
Thiamine Hydrochloride	0.001 g
Sodium Chloride	5 g
Dipotassium Phosphate	5 g
Manganese Chloride	0.14 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Sorbitan Monooleate Complex	0.2 g
Bacto Agar	15 g

Final pH 6.7 ± 0.2 at 25°C

User Quality Control

Identity Specifications

APT Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.12%, soluble in distilled or deionized water on boiling. Solution, upon cooling, is 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 6.12% Solution at 25°C:	pH 6.7 ± 0.2

APT Broth

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

Cultural Response

Prepare APT Agar and APT Broth per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus fermentum	9338	100-1,000	good
Lactobacillus viridescens	12706	100-1,000	good

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

APT Broth

Formula Per Liter

Bacto Yeast Extract	7.5 g
Bacto Tryptone	12.5 g
Bacto Dextrose	10 g
Sodium Citrate	5 g
Thiamine Hydrochloride	0.001 g
Sodium Chloride	5 g
Dipotassium Phosphate	5 g
Manganese Chloride	0.14 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Sorbitan Monooleate Complex	0.2 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.

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

APT Agar
APT Broth

Materials Required but not Provided

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

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   APT Agar: 61.2 grams;
   APT Broth: 46.2 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Avoid overheating.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For maintaining stock cultures of *Lactobacillus viridescens* ATCC® 12706 prepare a stab inoculation. Prepare stock cultures in triplicate at monthly intervals. One of the transfers is saved for the

preparation of stock cultures. The others are used to prepare inoculum in APT Broth for assay as needed. Following incubation at 35-37°C for 24-48 hours, store stock cultures at 2-8°C.

Results

Refer to appropriate references and procedures for results.

References

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

Packaging

APT Agar	500 g	0654-17
	2 kg	0654-07
	10 k	0654-08
APT Broth	500 g	0655-17

Bacto® Acetate Differential Agar

Intended Use

Bacto Acetate Differential Agar is used for differentiating microorganisms of the *Shigella* genus from those of the *Escherichia* genus.

Also Known As

Acetate Differential Agar is also known as Sodium Acetate Agar.

Summary and Explanation

Although classified taxonomically as different species for clinical reasons, *Shigella* species and *E. coli* are essentially the same genus and species. Their DNA relatedness is high, they are difficult to differentiate biochemically, and they cross-react serologically.[1] One way they can be differentiated is by using a medium containing sodium acetate as a sole source of carbon. Many strains of *E. coli* are able to use acetate as a carbon source, whereas typical cultures of *Shigella* are unable to grow.

Trabulsi and Ewing[2] developed Acetate Differential Agar by substituting sodium acetate for sodium citrate in their basal medium, Simmons Citrate Agar. They demonstrated that none of the *Shigella* tested grew on the Acetate Differential Agar. A large percentage of *E. coli* strains, belonging to various O antigen groups, did use the acetate within 2 to 7 days of incubation.

The majority of *Salmonella, Citrobacter, Klebsiella, Enterobacter* and *Serratia* groups use acetate and grow on Acetate Differential Agar within 1 to 7 days. *Proteus* and *Providencia* groups, however, fail to grow on the medium. Several standard methods list Acetate

User Quality Control

Identity Specifications

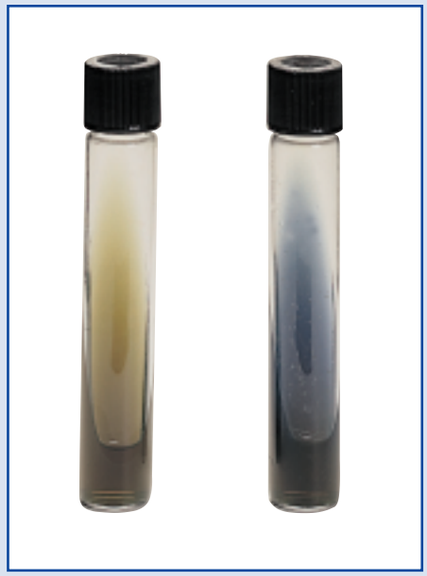
Dehydrated Appearance:	Medium yellowish-tan to light green, free-flowing, homogeneous.
Solution:	2.92% solution, soluble in distilled or deionized water on boiling. Solution is emerald green, slightly opalescent.
Prepared Medium:	Emerald green to green, slightly opalescent.
Reaction of 2.92% Solution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

Cultural Response

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

ORGANISMS	ATCC®	GROWTH	APPEARANCE
Escherichia coli	25922*	good	blue
Shigella sonnei	25931*	poor to good	green

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



Uninoculated tube — *Escherichia coli* ATCC® 25922

Differential Agar as a possible medium for the differentiation of *Enterobacteriaceae*.[2,3,4]

Principles of the Procedure

Acetate Differential Agar consists of a mixture of salts and sodium acetate as a sole source of carbon. Brom Thymol Blue is added to detect the alkaline products resulting from acetate utilization. Mono Ammonium Phosphate and Dipotassium Phosphate provide buffering capability. Bacto Agar is a solidifying agent.

Formula

Acetate Differential Agar

Formula Per Liter

Sodium Acetate	2 g
Magnesium Sulfate	0.1 g
Sodium Chloride	5 g
Mono Ammonium Phosphate	1 g
Dipotassium Phosphate	1 g
Bacto Brom Thymol Blue	0.08 g
Bacto Agar	20 g

Final pH 6.7 ± 0.1 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.

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

Acetate Differential Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
0.85% NaCl solution

Method of Preparation

1. Suspend 29.2 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense into tubes to allow a 10 mm butt and a 30 mm slant.
4. Autoclave at 121°C for 15 minutes.
5. Allow tubes to cool in a slanted position to give the recommended butt and slant size.

Test Procedure

1. Inoculate agar slant surfaces with 16-18 hour cultures emulsified in 1 ml of 0.85% sodium chloride solution.
2. Incubate aerobically at 35 ± 2°C for at least 7 days; read daily, examining for a change in the color of the medium from green to blue.

Results

Positive: Blue
Negative: Green

Limitations of the Procedure

1. Some strains of *E. coli* and nonmotile, anaerogenic *E. coli* (*Alkalescens-Dispar*) grow slowly or not at all and, thus, may give a false-negative reaction.
2. Further biochemical, physiological and serological tests are required to differentiate species.
3. False-positive results may occur from a too heavy inoculum.
4. MacFaddin[5] suggests that correct results occur only when some syneresis fluid is present in the bottom of the tube (junction of the slant and butt).

References

1. **Gray, L. D.** 1995. *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*, pp. 450-456. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
3. **Andrews, W. H., G. A. June, and P. S. Sherrod.** 1995. *Shigella*, p. 6.01-6.06. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 17-20. Williams & Wilkins, Baltimore, MD.

Packaging

Acetate Differential Agar	500 g	0742-17

Bacto® Actinomycete Isolation Agar
Bacto Glycerol

Intended Use

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

Bacto Glycerol is used in preparing microbiological culture media.

Summary and Explanation

Although some genera are important to human medicine, most of the actinomycetes are part of the indigenous flora of soil, water, and vegetation. Actinomycetes may impart a musty odor to water or a muddy flavor to fish.[2] Actinomycetes can cause massive growths which will form a thick foam in the activated sludge process, causing a disruption in wastewater treatment.[3,4] Actinomycetes are gram positive, acid-fast cells, growing as filaments that may branch and may form irregularly shaped rods and cocci.

Olsen[1] formulated Actinomycete Isolation Agar for isolating and cultivating actinomycetes from soil and water. The formula, supplemented with Glycerol, is a highly purified fermentable alcohol used occasionally for differentiating certain bacteria and in media for isolating and culturing fastidious bacteria.

Principles of the Procedure

Actinomycete Isolation Agar contains Sodium Caseinate which is a source of nitrogen. Asparagine is an amino acid and a source of organic nitrogen. Sodium Propionate is a substrate used in anaerobic fermentation. Dipotassium Phosphate provides buffering capability to maintain pH balance. Magnesium Sulfate and Ferrous Sulfate provide sources of sulfates and metallic ions. Bacto Agar is the solidifying agent. The added Glycerol is a source of carbon.

User Quality Control

Identity Specifications

Actinomycete Isolation Agar

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

Cultural Response

Prepare Actinomycete Isolation Agar per label directions with the addition of 0.5% Glycerol. Inoculate and incubate at 30 ± 2°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Streptomyces achromogenes	12767	100-1,000	good
Streptomyces albus	3004	100-1,000	good
Streptomyces lavendulae	8664	100-1,000	good

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

Formula

Actinomycete Isolation Agar

Formula Per Liter

Sodium Caseinate	2 g
Asparagine	0.1 g
Sodium Propionate	4 g
Dipotassium Phosphate	0.5 g
Magnesium Sulfate	0.1 g
Ferrous Sulfate	0.001 g
Bacto Agar	15 g

Final pH 8.1 ± 0.2 at 25°C

Glycerol

Not applicable

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.

   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 Glycerol 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

Actinomycete Isolation Agar
Glycerol

Materials Required but not Provided

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

Method of Preparation

1. Suspend 22 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 5 grams Glycerol.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs. Transport immediately to the laboratory, in accordance with recommended guidelines.
2. Process each specimen as appropriate for that specimen.

Test Procedure

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

Results

Refer to appropriate references and procedures for results.

References

1. **Olsen, E. H.** 1960. Personal Communication.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Lechevalier, H. A.** 1975. Actinomycetes of sewage-treatment plants. Environ. Protection Technol. Ser., EPA-600/2-75-031, U. S. Environmental Protection Agency, Cincinnati, OH.
4. **Lechevalier, M. P., and H. A. Lechevalier.** 1974. *Nocardia amarae*, sp. nov., an actinomycete common in foaming activated sludge. Int. J. Syst. Bacteriol. **24**:278.

Packaging

Actinomycete Isolation Agar	100 g	0957-15
	500 g	0957-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Agar Medium No. F

Intended Use

Bacto Agar Medium No. F is a selective medium used for detecting *Enterobacteriaceae* and other gram-negative bacteria in pharmaceutical products.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.15% solution, soluble in distilled or deionized water on boiling. Solution is reddish-purple, slightly opalescent.
Prepared Medium:	Reddish-purple, slightly opalescent without a precipitate.
Reaction of 5.15% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Agar Medium No. F per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	COLONY DESCRIPTION
Escherichia	11775	100-1,000	good	reddish-purple, may have a slight precipitate around the colonies
Salmonella gallinarum	9184	100-1,000	good	reddish-purple, may have a slight precipitate around the colonies
Staphylococcus aureus	6538	1,000-2,000	inhibited	–

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

Summary and Explanation

Agar Medium No. F is based on the formula for Agar Medium F (Agar Medium with Bile, Crystal Violet, Neutral Red and Glucose) described in DAB, 10th Edition. Agar Medium No. F is recommended for use in the detection of *Enterobacteriaceae* and other gram-negative bacteria in pharmaceuticals.[1]

Principles of the Procedure

Agar Medium No. F, based on Violet Red Bile Agar and Violet Red Bile Glucose Agar, uses Sodium Cholate instead of the Bile Salts No. 3 used in Violet Red Bile Agar and Violet Red Bile Glucose Agar. Carbon and nitrogen sources required for growth of a variety of organisms are provided by Bacto Peptone and Yeast Extract. Selectivity is due to the presence of Crystal Violet and Sodium Cholate which markedly to completely inhibit growth of gram-positive microorganisms. Bacto Agar is the solidifying agent.

Differentiation is based on the fermentation of Dextrose and Lactose. Organisms growing in this medium that can ferment dextrose, such as members of the family *Enterobacteriaceae*, produce a localized pH drop which, followed by absorption of the Neutral Red, imparts a reddish-purple color to the colony. A zone of precipitated Sodium Cholate may also be present due to this drop in pH. These reactions are further intensified in those organisms that can ferment both lactose and dextrose.

Formula

Agar Medium No. F

Formula Per Liter

Bacto Peptone	7 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Bacto Dextrose	10 g

Sodium Chloride	5 g
Sodium Cholate	1.5 g
Neutral Red	0.03 g
Crystal Violet	0.002 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

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

Storage

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

Agar Medium No. F

Materials Required But Not Provided

Lactose Broth
Enterobacteriaceae Enrichment Broth Mossel (EE Broth Mossel)
Flasks with closures
Distilled or deionized water
Incubator (35°C)
Polysorbate 20 or Polysorbate 80

Method of Preparation

1. Suspend 51.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Sterilize by steaming for 30 minutes. Do Not Autoclave.

Specimen Collection and Preparation

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

Test Procedure[1,2]

1. Pre-enrich the sample in Lactose Broth. If the sample is insoluble in water, add 0.1 ml of polysorbate 20 or polysorbate 80 to the Lactose Broth.
2. Homogenize the mixture and incubate at 35 ± 2°C for 2-5 hours.
3. Transfer 1 ml of enriched Lactose Broth to 100 ml of EE Broth Mossel (Enterobacteriaceae Enrichment Broth-Mossel).
4. Incubate at 35 ± 2°C for 24-48 hours.
5. Subculture all enrichment broth cultures showing growth onto Agar Medium No. F.
6. Incubate at 35 ± 2°C for 18-24 hours.
7. Examine plates for the presence of presumptive *Enterobacteriaceae* colonies.

Results

Colonies of the family *Enterobacteriaceae* are reddish-purple in color and are generally surrounded by a zone of precipitated bile salt. Growth of gram-positive organisms is markedly to completely suppressed. Further biochemical testing is necessary to confirm the presence and identification of *Enterobacteriaceae*. Consult appropriate references for further information on identification of *Enterobacteriaceae*.[3,4]

References

1. **DAB, 10th Edition.** 1991. V.2 Biology, V.2.1.8 Proving Certain Microorganisms, VIII.10 Media (Microbiological Pollution), Frankfurt/Main.
2. **British Pharmacopoeia, Volume II, Appendix XVI.** 1988. HMSO, London.
3. **Farmer, J. J.** 1995. *Enterobacteriaceae*: introduction and identification. *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. **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

Agar Medium No. F	500 g	0666-17

Amino Acid Assay Media

Bacto® Lysine Assay Medium · Bacto Methionine Assay Medium Bacto Cystine Assay Medium

Intended Use

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

Bacto Methionine Assay Medium is used for determining methionine concentration by the microbiological assay technique.

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

Also Known As

Lysine Assay Medium, Methionine Assay Medium and Cystine Assay Medium are also referred to as Amino Acid Assay Media.

Summary and Explanation

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

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

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

Principles of the Procedure

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

Formula

Lysine Assay Medium, Methionine Assay Medium, or Cystine Assay Medium

All amino acid assay media contain the following formula. Omit the particular amino acid to be assayed from the medium.

Formula Per Liter		
Bacto Dextrose	50	g
Sodium Acetate	40	g
Ammonium Chloride	6	g
Monopotassium Phosphate	1.2	g
Dipotassium Phosphate	1.2	g
Magnesium Sulfate	0.4	g
Ferrous Sulfate	20	mg
Manganese Sulfate	40	mg
Sodium Chloride	20	mg
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Xanthine	20	mg
Thiamine Hydrochloride	1	mg
Pyrodoxine Hydrochloride	2	mg
Pyridoxamine Hydrochloride	600	mg
Pyridoxal Hydrochloride	600	mg
Calcium Pantothenate	1	mg
Riboflavin	1	mg
Nicotinic Acid	2	mg
p-Aminobenzoic Acid	200	µg
Biotin	2	µg
Folic Acid	20	µg
Glycine	0.2	g
DL-Alanine	0.4	g
Bacto Asparagine	0.8	g
L-Aspartic Acid	0.2	g
L-Proline	0.2	g
DL-Serine	0.1	g
DL-Tryptophane	80	mg
L-Cystine	0.1	g
L-Glutamic Acid	0.6	g
L-Histidine Hydrochloride	0.124	g
DL-Phenylalanine	0.2	g
DL-Threonine	0.4	g
L-Tyrosine	0.2	g
DL-Valine	0.5	g
L-Lysine Hydrochloride	0.5	g
DL-Methionine	0.2	g
DL-Isoleucine	0.5	g
DL-Leucine	0.5	g
L-Arginine Hydrochloride	0.484	g

Final pH 6.7 ± 0.2 at 25ºC

User Quality Control

Identity Specifications

Lysine Assay Medium, Methionine Assay Medium, or Cystine Assay Medium

Dehydrated Appearance:	White to off-white, homogeneous, may have a tendency to clump.
Solution:	5.25% (single strength) and 10.5% (double strength) solution, soluble in distilled or deionized water upon boiling. Solution (single strength) is light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Single strength-light to medium amber, clear.
Reaction of 5.25% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Lysine Assay Medium, Methionine Assay Medium and Cystine Assay Medium per label directions. These media will support the growth of *Pediococcus acidilactici* ATCC® 8042 when supplemented with the appropriate amino acid. Test Lysine Assay Medium by creating a standard curve using L-Lysine at 0 to 300 µg per 10 ml. Test Methionine Assay Medium by creating a standard curve using DL-Methionine at 0 to 60 µg per 10 ml. Test Cystine Assay Medium by creating a standard curve using L-Cystine at 0 to 50 µg per 10 ml.

The test organism listed is the minimum used for performance testing.

Precautions

1. For Laboratory Use.
2. Great care to avoid contamination of media or glassware must be taken in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware is heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. **Methionine Assay Medium and Cystine Assay Medium**

   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): 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.

4. Take precautions to keep sterilizing and cooling conditions uniform throughout the assay.
5. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated media at 2-8ºC. The dehydrated medium is very hygroscopic and may be stored in a container with calcium chloride or other desiccant. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Lysine Assay Medium or
Methionine Assay Medium or
Cystine Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Pediococcus acidilactici* ATCC® 8042
Sterile tubes, optically standardized
Centrifuge
Spectrophotometer (660 nm)
L-Lysine HCl
DL-Methionine
L-Cystine
Sterile 0.85% NaCl

Method of Preparation

Lysine Assay Medium, Methionine Assay Medium, and Cystine Assay Medium

1. Suspend 10.5 grams in 100 ml distilled or deionized water.
2. Boil for 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

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

Test Procedure

Stock Culture and Inoculum

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

The inoculum for assay is prepared by subculturing the test organism into 10 ml Lactobacilli Broth AOAC or Micro Inoculum Broth. Incubate at 35-37ºC for 16-24 hours. After incubation, centrifuge the cells under aseptic conditions and decant the liquid supernatant. Wash the cells 3 times with 10 ml sterile 0.85% NaCl solution. After the third wash, resuspend the cells in 10 ml sterile 0.85% NaCl solution. Dilute the 10 ml cell suspension with the appropriate amount of sterile 0.85% NaCl solution. (See Table 1 below.) One drop of the diluted inoculum suspension is used to inoculate each of the assay tubes.

Amino Acid Solution

Prepare stock solutions of each amino acid as described in Table 1. If the DL form is used, twice the concentration of the amino acid is required. Prepare the stock solutions fresh daily.

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

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

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

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.

Table 1. *Preparation of inoculum dilution, amino acid stock and working solution.*

ASSAY MEDIUM	TEST CULTURE	PREPARATION OF INOCULUM DILUTION (CELL SUSPENSION + (STERILE 0.85% NaCl)	PREPARATION OF AMINO ACID STOCK SOLUTION (AMINO ACID)	+ (DISTILLED H_2O)	STANDARD WORKING SOLUTION (STOCK SOLUTION) + (DISTILLED H_2O)	VOLUME OF STANDARD WORKING SOLUTION (ml/10 ml TUBE)	FINAL AMINO ACID CONCENTRATION µg/10 ml
Cystine Assay Medium	*Pediococcus acidilactici* ATCC® 8042	1 ml + 19 ml	L-cystine	1 g + 100 ml + 1 ml HCl heated, then cooled, add up to 1,000 ml	1 ml + 99 ml	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 5, 10, 15, 20, 25, 30, 40, 50
Lysine Assay Medium	*Pediococcus acidilactici* ATCC® 8042	1 ml + 19 ml	L-lysine	6 g + 1,000 ml	1 ml + 99 ml	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 30, 60, 90, 120, 150, 180, 240, 300
Methionine Assay Medium	*Pediococcus acidilactici* ATCC® 8042	1 ml + 19 ml	DL-methionine	1.2 g + 1,000 ml	1 ml + 99 ml	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 6, 12, 18, 24, 30, 36, 48, 60

2. Determine the amount of amino acid at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of amino acid in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

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

References

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

Packaging

Lysine Assay Medium	100 g	0422-15*
Methionine Assay Medium	100 g	0423-15*
Cystine Assay Medium	100 g	0467-15*

*Store at 2-8°C

Bacto® Anaerobic Agar

Intended Use

Bacto Anaerobic Agar is used for cultivating anaerobic microorganisms.

Summary and Explanation

Brewer[1] described a special Petri dish cover that allowed surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on an agar-based medium having a low oxidation-reduction potential. Anaerobic Agar is a modification of Brewer's original formula. This medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.[2,3,4]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in distilled or deionized water on boiling. Light amber, slightly opalescent. As the medium cools, it becomes green due to aeration.
Prepared Medium:	Light green, slightly opalescent.
Reaction of 5.8% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis	25285*	100-1,000	good
Clostridium perfringens	13124*	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.

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

Principles of the Procedure

Casitone provides the nitrogen, vitamins and amino acids in Anaerobic Agar. Dextrose is a carbon source. Sodium Chloride maintains the osmotic equilibrium. Sodium Thioglycollate and Sodium Formaldehyde Sulfoxylate are reducing agents. Methylene Blue serves as an indicator of anaerobiosis with a blue color indicating the presence of oxygen. Bacto Agar is the solidifying agent.

Formula

Anaerobic Agar

Formula Per Liter

Bacto Casitone	20 g
Sodium Chloride	5 g
Bacto Dextrose	10 g
Bacto Agar	20 g
Sodium Thioglycollate	2 g
Sodium Formaldehyde Sulfoxylate	1 g
Methylene Blue	0.002 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 material.

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

Anaerobic Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 58 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense as desired.

Specimen Collection and Preparation

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[2] Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

Standard Petri Dishes:[2]

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

Brewer Anaerobic Agar Plates:

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

For a complete discussion on anaerobic and microaerophilic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[2,3,4] For the examination of anaerobic bacteria in food, refer to Standard Methods.[7,8,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[2]
3. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[2]
4. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure that the organism is an anaerobe.[2]
5. Methylene blue is toxic to some anaerobic bacteria.

References

1. **Brewer, J. H.** 1942. A new Petri dish and technique for use in the cultivation of anaerobes and microaerophiles. Science **95**:587.
2. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Etiological agents recovered from clinical material, p. 474-503. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
6. **Smith, L. D. S.** 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Il.
7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithesburg, MD.
8. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
9. **Marshall, R. T. (ed.).** 1992. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Anaerobic Agar	500 g	0536-17

Bacto® Antibiotic Assay Media

Bacto Antibiotic Medium 1 · Bacto Antibiotic Medium 2 Bacto Antibiotic Medium 3 · Bacto Antibiotic Medium 4 Bacto Antibiotic Medium 5 · Bacto Antibiotic Medium 8 Bacto Antibiotic Medium 9 · Bacto Antibiotic Medium 10 Bacto Antibiotic Medium 11 · Bacto Antibiotic Medium 12 Bacto Antibiotic Medium 19

Intended Use

Bacto Antibiotic Assay Media are used for determining antibiotic potency by the microbiological assay technique.[1,6,7]

User Quality Control

Identity Specifications

Antibiotic Medium 1

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.05% solution, soluble in distilled or deionized water upon boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.05% Solution at 25°C:	pH 6.55 ± 0.05

Antibiotic Medium 2

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
Solution:	2.55% solution, soluble in distilled or deionized water upon boiling; light- medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light-medium amber, slightly opalescent.
Reaction of 2.55% Solution at 25°C:	pH 6.55 ± 0.05

Antibiotic Medium 3

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	1.75% solution, soluble in distilled or deionized water; light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 1.75% Solution at 25°C:	pH 7.0 ± 0.05

continued on following page

Also Known As

DIFCO PRODUCT NAME	GROVE AND RANDALL[8]	USP[1]	21 CFR[6]	AOAC[7]
Antibiotic Medium 1	Penassay Seed Agar	Medium 1	Medium 1	Agar Medium A
Antibiotic Medium 2	Penassay Base Agar	Medium 2	Medium 2	Agar Medium C
Antibiotic Medium 3	Penassay Broth	Medium 3	Medium 3	Broth Medium A
Antibiotic Medium 4	Yeast Beef Agar	–	Medium 4	Agar Medium B
Antibiotic Medium 5	Streptomycin Assay Agar	Medium 5	Medium 5	Agar Medium E
Antibiotic Medium 8	–	Medium 8	Medium 8	Agar Medium D
Antibiotic Medium 9	Polymyxin Base Agar	Medium 9	Medium 9	–
Antibiotic Medium 10	Polymyxin Seed Agar	Medium 10	Medium 10	–
Antibiotic Medium 11	Neomycin Assay Agar	–	Medium 11	Agar Medium J
Antibiotic Medium 12	–	–	–	–
Antibiotic Medium 19	–	Medium 19	Medium 19	–

Summary and Explanation

The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.[1] Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.[1] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al.[2] for the assay of penicillin, was later modified by Foster and Woodruff[3] and by Schmidt and Moyer[4] et al.

Antibiotic Assay Media are prepared according to the specifications of the U.S. Pharmacopeia (USP) XXIII[1], European Pharmacopeia, Code of Federal Regulations (21CFR[6]) and the Association of Official Analytical Chemists (AOAC)[7]. The Antibiotic Media are identified numerically and also, where applicable, with names assigned by Grove and Randall in Assay Methods of Antibiotics.[8] Antibiotic Medium 19 corresponds to the use described in Outline of Details for Official Microbiological Assays of Antibiotics.[9]

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

Principles of the Procedure

Cylinder Plate Assay

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

Turbidimetric Assay

The turbidimetric method is based on the inhibition of growth of a microbial culture in a fluid medium containing a uniform solution of an antibiotic.[1] Turbidimetric determinations have the advantage of requiring a short incubation period, providing test results after 3 or 4 hours. However, the presence of solvents or other inhibitory materials may influence turbidimetric assays more markedly than cylinder plate assays. Use of this method is appropriate only when test samples are clear.

User Quality Control cont.

Antibiotic Medium 4

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.65% solution, soluble in distilled or deionized water on boiling; light amber, very slightly opalescent.
Prepared Medium:	Light amber, very slightly to slightly opalescent.
Reaction of 2.65% Solution at 25°C:	pH 6.55 ± 0.05

Antibiotic Medium 5

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.55% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 2.55% Solution at 25°C:	pH 7.9 ± 0.1

Antibiotic Medium 8

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.55% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 2.55% Solution at 25°C:	pH 5.85 ± 0.05

Antibiotic Medium 9

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water upon boiling; light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent with slight flocculent precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.25 ± 0.05

Antibiotic Medium 10

Dehydrated Appearance:	Beige, homogeneous, moist with a tendency to clump.
Solution:	5.2% solution, soluble in distilled or deionized water upon boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 5.2% Solution at 25°C:	pH 7.25 ± 0.05

Antibiotic Medium 11

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.05% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.05% Solution at 25°C:	pH 8.0 ± 0.1

Antibiotic Medium 12

Dehydrated Appearance:	Tan, homogeneous, free-flowing.
Solution:	6.25% solution, soluble in distilled or deionized water upon boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 6.25% Solution at 25°C:	pH 6.1 ± 0.1

Antibiotic Medium 19

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
Solution:	6.0% solution, soluble in distilled or deionized water upon boiling; medium amber, very slightly to slightly opalescent.
Prepared Medium:	Medium amber, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 6.1 ± 0.1

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User Quality Control cont.

Cultural Response

Antibiotic Medium 1
Antibiotic Medium 2
Prepare Antibiotic Medium 1 or Antibiotic Medium 2 per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Staphylococcus aureus	6538P	30-300	good

Antibiotic Medium 3
Prepare Antibiotic Medium 3 per label directions. Inoculate and incubate at 35 ± 2°C for up to 24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Enterococcus faecium	10541	approx. 10^7	good
Escherichia coli	10536	approx. 10^7	good
Klebsiella pneumoniae	10031	approx. 10^7	good
Staphylococcus aureus	6538P	approx. 10^7	good

Antibiotic Medium 4
Prepare Antibiotic Medium 4 per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Micrococcus luteus	9341	30-300	good

Antibiotic Medium 5
Antibiotic Medium 8
Prepare Antibiotic Medium 5 or Antibiotic Medium 8 per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Bacillus subtilis	6633	30-300	good

Antibiotic Medium 9
Antibiotic Medium 10
Prepare Antibiotic Medium 9 or Antibiotic Medium 10 per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Bordetella bronchiseptica	4617	30-500	good

Antibiotic Medium 11
Prepare Antibiotic Medium 11 per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Micrococcus luteus	9341	30-300	good
Staphylococcus epidermidis	12228	30-300	good

Antibiotic Medium 12
Antibiotic Medium 19
Prepare Antibiotic Medium 12 or Antibiotic Medium 19 per label directions. Inoculate and incubate at 30 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Saccharomyces cerevisiae	2601	30-300	good

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

*When tested in an appropriate antibiotic assay procedure in parallel with a previously approved lot of material, inhibition of growth should produce the specified zones and be comparable to the previously approved lot.[6]

Formula

Antibiotic Medium 1 (Penassay Seed Agar)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Casitone	4 g
Bacto Peptone	6 g
Bacto Dextrose	1 g
Bacto Agar	15 g

Final pH 6.55 ± 0.05 at 25°C

Antibiotic Medium 2 (Penassay Base Agar)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Peptone	6 g
Bacto Agar	15 g

Final pH 6.55 ± 0.05 at 25°C

Antibiotic Medium 3 (Penassay Broth)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	1.5 g
Bacto Peptone	5 g
Bacto Dextrose	1 g
Sodium Chloride	3.5 g
Dipotassium Phosphate	3.68 g
Monopotassium Phosphate	1.32 g

Final pH 7.0 ± 0.05 at 25°C

Antibiotic Medium 4 (Yeast Beef Agar)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Peptone	6 g
Bacto Dextrose	1 g
Bacto Agar	15 g

Final pH 6.55 ± 0.05 at 25°C

Antibiotic Medium 5 (Streptomycin Assay Agar)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Peptone	6 g
Bacto Agar	15 g

Final pH 7.9 ± 0.1 at 25°C

Antibiotic Medium 8

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Peptone	6 g
Bacto Agar	15 g

Final pH 5.85 ± 0.05 at 25°C

Antibiotic Medium 9 (Polymyxin Base Agar)

Formula Per Liter

Bacto Casitone	17 g
Soytone	3 g
Bacto Dextrose	2.5 g

Sodium Chloride ..... 5 g
Dipotassium Phosphate ..... 2.5 g
Bacto Agar ..... 20 g
Final pH 7.25 ± 0.05 at 25°C

Antibiotic Medium 10 (Polymyxin Seed Agar)
Formula Per Liter
Bacto Casitone ..... 17 g
Soytone ..... 3 g
Bacto Dextrose ..... 2.5 g
Sodium Chloride ..... 5 g
Dipotassium Phosphate ..... 2.5 g
Bacto Agar ..... 12 g
Polysorbate 80 ..... 10 g
Final pH 7.25 ± 0.05 at 25°C

Antibiotic Medium 11 (Neomycin Assay Agar)
Formula Per Liter
Bacto Beef Extract ..... 1.5 g
Bacto Yeast Extract ..... 3 g
Bacto Casitone ..... 4 g
Bacto Peptone ..... 6 g
Bacto Dextrose ..... 1 g
Bacto Agar ..... 15 g
Final pH 7.95 ± 0.05 at 25°C

Antibiotic Medium 12
Formula Per Liter
Bacto Beef Extract ..... 2.5 g
Bacto Yeast Extract ..... 5 g
Bacto Peptone ..... 10 g
Bacto Dextrose ..... 10 g
Sodium Chloride ..... 10 g
Bacto Agar ..... 25 g
Final pH 6.1 ± 0.1 at 25°C

Antibiotic Medium 19
Formula Per Liter
Bacto Peptone ..... 9.4 g
Bacto Beef Extract ..... 2.4 g
Bacto Yeast Extract ..... 4.7 g
Bacto Dextrose ..... 10 g
Sodium Chloride ..... 10 g
Bacto Agar ..... 23.5 g
Final pH 6.1 ± 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 Antibiotic Media (except Antibiotic Medium 10) below 30°C. Store dehydrated Antibiotic Medium 10 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 directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Antibiotic Medium 1
Antibiotic Medium 2
Antibiotic Medium 3
Antibiotic Medium 4
Antibiotic Medium 5
Antibiotic Medium 8
Antibiotic Medium 9
Antibiotic Medium 10
Antibiotic Medium 11
Antibiotic Medium 12
Antibiotic Medium 19

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Sterile tubes
Waterbath
Test organisms
Maintenance medium for test organisms
Cylinder Plate Assay: Petri dishes 20 x 100 mm with suitable covers
Stainless steel or porcelain cylinders
Turbidimetric Assay: Glass or plastic tubes

Selection of Media for the Microbiological Assay of Antibiotics[1,6]

Antibiotic	Assay Method	Organism	ATCC®	Maintenance Medium	Inoculum Medium	Cylinder Plate Base Layer	Cylinder Plate Seed Layer	Turbidimetric Assay Medium
Amikacin	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Amoxicillin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Amphotericin B	Cylinder Plate	*Saccharomyces cerevisiae*	9763	19	19		19	
Ampicillin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Bacitracin	Cylinder Plate	*Micrococcus luteus*	7468	1	1	2	1	
Bacitracin	Cylinder Plate	*Micrococcus luteus*	10240**	1	1	1	1	
Capreomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3

continued on following page

Selection of Media for the Microbiological Assay of Antibiotics[1,6] cont.

Antibiotic	Assay Method	Organism	ATCC®	Maintenance Medium	Inoculum Medium	Cylinder Plate Base Layer	Cylinder Plate Seed Layer	Turbidimetric Assay Medium
Carbenicillin	Cylinder Plate	*Pseudomonas aeruginosa*	25619	1	1	9	10	
Cefaclor	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefadroxil	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefamandole	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefazolin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefotaxime	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefoxitin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephalexin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephaloglycin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephaloridine	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephalothin	Cylinder Plate	*Staphylococcus aureus*	6538P*	1	1	2	1	
Cephapirin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephradine	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Chloramphenicol	Turbidimetric	*Escherichia coli*	10536	1	1			3
Chlortetracycline	Cylinder Plate	*Bacillus cereus*	11778**	1		8	8	
Chlortetracycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Chlortetracycline	Turbidimetric	*Staphylococcus aureus*	9144**		3			3
Clindamycin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Cloxacillin	Cylinder Plate	*Staphylococcus aureus*	6538P*	1	1	2	1	
Colistimethate, sodium	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	1	9	10	
Colistin	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	1	9	10	
Cyclacillin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Cycloserine	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Dactinocycin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	5	5	
Demeclyocycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Dicloxacillin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Dihydro-	Cylinder Plate	*Bacillus subtilis*	6633	1	1	5	5	
streptomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3
Doxycycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Erythromycin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Erythromycin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3		11	
Gentamicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Gramicidin	Turbidimetric	*Enterococcus faecium*	10541	3	3			3
Hygromycin B	Cylinder Plate	*Bacillus subtilis*	6633**			5	5	
Kanamycin	Turbidimetric	*Staphylococcus aureus*	6538P	1	1			3
Kanamycin B	Cylinder Plate	*Bacillus subtilis*	6633	1	1	5	5	
Lincomycin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3	5	11	
Lincomycin	Turbidimetric	*Staphylococcus aureus*	6538P	1	1			3
Meclocycline	Turbidimetric	*Staphylococcus aureus*	6538P	1	1			3
Methacycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Methicillin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	

continued on following page

Selection of Media for the Microbiological Assay of Antibiotics[1,6] cont.

Antibiotic	Assay Method	Organism	ATCC®	Maintenance Medium	Inoculum Medium	Cylinder Plate Base Layer	Cylinder Plate Seed Layer	Turbidimetric Assay Medium
Mitomycin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	8	8	
Nafcillin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Natamycin	Cylinder Plate	*Saccharomyces cerevisiae*	9763	19	19		19	
Neomycin	Cylinder Plate	*Staphylococcus aureus*	6538P***	1	1	11	11	
Neomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3
Netilmicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Novobiocin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3	2	2	
Novobiocin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	2	1	
Nystatin	Cylinder Plate	*Saccharomyces cerevisiae*	2601	19	19		19	
Oleandomycin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3		11	
Oleandomycin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Oxacillin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Oxytetracyline	Cylinder Plate	*Bacillus cereus*	11778**	1			8	
Oxytetracycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Paromomycin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Penicillin G	Cylinder Plate	*Staphylococcus aureus*	6538P*	1	1	2	1	
Penicillin V	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Plicomycin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	8	8	
Polymyxin B	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	1	9	10	
Procaine Penicillin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3	1	4	
Rifampin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	2	2	
Rolitetracycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Sisomicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Spectinomycin	Turbidimetric	*Escherichia coli*	10536	1	1			3
Streptomycin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	5	5	
Streptomycin	Cylinder Plate	*Bacillus subtilis*	6633**	32		5	5	
Streptomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3
Tetracycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Tobramycin	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Troleandomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3
Tyrothricin	Turbidimetric	*Enterococcus faecium*	10541	3	3			3
Vancomycin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	8	8	

* For USP methods, use *Staphylococcus aureus* ATCC® 29737.
** Specified by AOAC for Drugs in Feeds.
*** For USP methods, use *Staphylococcus epidermidis* ATCC® 12228.

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:
   Antibiotic Medium 1 - 30.5 grams;
   Antibiotic Medium 2 - 25.5 grams;
   Antibiotic Medium 3 - 17.5 grams;
   Antibiotic Medium 4 - 26.5 grams;
   Antibiotic Medium 5 - 25.5 grams;
   Antibiotic Medium 8 - 25.5 grams;
   Antibiotic Medium 9 - 50 grams;
   Antibiotic Medium 10 - 52 grams;
   Antibiotic Medium 11 - 30.5 grams;
   Antibiotic Medium 12 - 62.5 grams;
   Antibiotic Medium 19 - 60 grams.
2. Boil to dissolve completely (except Antibiotic Medium 3, which dissolves without boiling).

3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45-50°C.
5. Antibiotic Medium 11, only: To alter the pH, add 1N HCl or 1N NaOH to the medium at 45-50°C.
6. Dispense as appropriate.

Specimen Collection and Preparation

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

Test Organism Preparation

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

When *Bacillus subtilis* is used as the test organism, inoculate it on Antibiotic Medium 1 and incubate at 37°C for 1 week, wash spores from the agar surface, and heat the spores at 56°C for 30 minutes. Wash the spores 3 times in distilled water, heat again at 65°C for 30 minutes, and then dilute to the optimal concentration. This inoculum preparation should produce a sharp zone in the assay.

Antibiotic Medium modified by the addition of 300 mg manganese sulfate ($MnSO_4 \cdot H_2O$) per liter often aids the sporulation of *B. subtilis* and may be used in preparing the spore suspension. A standardized spore suspension prepared from *B. subtilis* ATCC® 6633 is available as Bacto Subtilis Spore Suspension.

When *B. cereus var. mycoides* is required, inoculate the organism on Antibiotic Medium 1 and incubate at 30°C for 1 week. Wash and prepare the spores as for *B. subtilis*, above. A standardized spore suspension of *B. cereus var. mycoides* is available as Bacto Cereus Spore Suspension.

Cylinder Plate Assay

Use 20 x 100 mm Petri dishes with sufficient depth so that cylinders used in the assay will not be pushed into the medium by the cover. Porcelain covers glazed on the outside, only, are recommended.

Use stainless steel or porcelain assay cylinders having the following dimensions (± 0.1 mm): 8 mm outside diameter, 6 mm inside diameter and 10 mm long.[1] Carefully clean the cylinders to remove all residues, using an occasional acid bath, i.e., with approximately 2N nitric acid or with chromic acid.[1] Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius.

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

Turbidimetric Assay

Use glass or plastic test tubes (i.e., 16 x 125 mm or 18 x 150 mm) that are relatively uniform in length, diameter and thickness and substantially free from surface blemishes.[1] Tubes that will be placed in the spectrophotometer should be matched and free of scratches or blemishes.[1] Clean the tubes thoroughly to remove all antibiotic residues and traces of cleaning solution and, prior to subsequent use, sterilize tubes that have been previously used.[1]

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

For a complete discussion of antibiotic assay methods, refer to appropriate procedures outlined in the references.[1,5,6,7]

Results

Refer to appropriate procedures for results.[1,5,6,7]

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. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. Biological Tests and Assays, p. 1690-1696. The United States Pharmacopeial Convention, Rockville, MD.
2. **Abraham.** 1941. Lancet. **2**:177.
3. **Foster and Woodruff.** 1943. J. Bacteriol. **46**:187.
4. **Schmidt, W. H., and A. J. Moyer**. 1944. Penicillin. I. Methods of assay. J Bacteriol. **47**:199.
5. **European Pharmacopoeia.** 1994. Council of Europe, 2nd ed. Maisonneuve S. A. Sainte-Ruffine, FR.
6. **Federal Register.** 1992. Tests and methods of assay of Antibiotics and Antibiotic-Containing Drugs. Fed. Regist. **21**:436.100-436.106.
7. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
8. **Grove, D. C., and W. A. Randall.** 1955. Assay methods of antibiotics. Medical Encyclopedia Inc., New York, NY.
9. **Kirshbaum, A., and B. Arret.** 1967. Outline of details for official microbiological assays of antibiotics. J. Pharm. Sci. **56**:512.

Packaging

Antibiotic Medium 1	500 g	0263-17
	2 kg	0263-07
	10 kg	0263-08
Antibiotic Medium 2	500 g	0270-17
	10 kg	0270-08
Antibiotic Medium 3	500 g	0243-17
	2 kg	0243-07
Antibiotic Medium 4	500 g	0244-17
Antibiotic Medium 5	500 g	0277-17
Antibiotic Medium 8	500 g	0667-17
Antibiotic Medium 9	500 g	0462-17
Antibiotic Medium 10	500 g	0463-17
Antibiotic Medium 11	500 g	0593-17
Antibiotic Medium 12	500 g	0669-17
Antibiotic Medium 19	500 g	0043-17

Bacto® Aseptic Commissioning Medium

Intended Use

Bacto Aseptic Commissioning Medium is a fluid medium used in validating aseptic packing lines.

Summary and Explanation

Aseptic Commissioning Medium is a basic medium in which growth can be demonstrated by either acid or gas production. It is ideally suited for validating and commissioning aseptic packing and filling lines.

Principles of Procedure

Peptone and Yeast Extract provide basic nutrients. Sucrose is a carbohydrate source. Phenol Red is a pH indicator. Sodium Chloride maintains the osmotic balance.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige to pink, free-flowing, homogeneous.
Solution:	1.75% solution, soluble in distilled or deionized water on warming, orange-red, clear.
Prepared Medium:	Orange-red, clear.
Reaction of 1.75% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare the medium per label directions. Inoculate test organisms into tubes with fermentation vials and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID	GAS
Bacillus cereus	14579	100-1,000	good	+	–
Enterobacter aerogenes	13048*	100-1,000	good	+	+
Escherichia coli	25922*	100-1,000	good	+**	+ or –
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 Bactrol Disks Technical Information.

**May revert to alkaline after prolonged incubation.

Formula

Aseptic Commissioning Medium

Formula Per Liter

Peptone	5 g
Yeast Extract	2.5 g
Sucrose	5 g
Phenol Red	5 mg
Sodium Chloride	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Aseptic Commissioning Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water

Method of Preparation

1. Suspend 17.5 grams in 1 liter distilled or deionized water.
2. Heat gently to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Test Procedure

1. Dispense reconstituted medium into the packing line upstream of the sterilization process.
2. Incubate final packs at 30°C, as appropriate, for up to 7 days.

Results

Gas production is demonstrated by swelling of the pack and acid production by a color change of the medium to yellow. Growth is indicated by turbidity in the medium.

Packaging

Aseptic Commissioning Medium	500 g	1862-17
	5 kg	1862-03

Bacto® Azide Blood Agar Base

Intended Use

Bacto Azide Blood Agar Base is used for isolating streptococci and staphylococci; for use with blood in determining hemolytic reactions.

Also Known As

"Blood Agar Base" may be abbreviated as BAB.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in distilled or deionized water upon boiling. Light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent without precipitate.
	With 5% blood, cherry red, opaque.
Reaction of 3.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Azide Blood Agar Base per label directions, enrich with 5% sterile defibrinated blood. Inoculate prepared medium and incubate at 35 ± 2°C. Read plates for growth, hemolysis, colony size at 18-24 and 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HEMOLYSIS
Enterococcus faecalis	19433*	100-1,000	good	alpha/ gamma
Escherichia coli	25922*	1,000-2,000	inhibited	–
Staphylococcus aureus	25923*	100-1,000	good	beta
Staphylococcus epidermidis	12228*	100-1,000	good	gamma
Streptococcus pneumoniae	6305	100-1,000	good	alpha
Streptococcus pyogenes	19615*	100-1,000	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.

Summary and Explanation

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

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

Principles of the Procedure

Tryptose and Beef Extract provide nitrogen, vitamins, carbon and amino acids. Sodium Chloride maintains osmotic balance. Sodium Azide is the selective agent, suppressing the growth of gram negative bacteria. Bacto Agar is the solidifying agent.

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

Formula

Azide Blood Agar Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Sodium Chloride	5 g
Sodium Azide	0.2 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep

container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

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

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Azide Blood Agar Base

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 33 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. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs. Transport immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions demonstrating both oxygen-stable and oxygen-labile streptolysins.[6]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.

Results

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

a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.

b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.

c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.

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

Limitations of the Procedure

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

References

1. **Edwards, S. J.** 1933. The diagnosis of *Streptococcus mastitis* by cultural methods. J. Comp. Pathol. Ther. **46**:211.
2. **Snyder, M. L., and H. C. Lichstein.** 1940. Sodium azide as an inhibition substance of gram-negative bacteria. J. Infect. Dis. **67**:113.
3. **Lichstein, H. C., and M. L. Snyder.** 1941. The inhibition of the spreading growth of *Proteus* and other bacteria to permit the isolation of associated streptococci. J. Bacteriol. **42**:653.
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6. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Azide Blood Agar Base	500 g	0409-17
	10 kg	0409-08

Bacto® Azide Dextrose Broth

Intended Use

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

Summary and Explanation

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

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

Principles of the Procedure

Azide Dextrose Broth contains Beef Extract and Tryptose as sources of carbon, nitrogen, vitamins and minerals. Dextrose is a fermentable carbohydrate. Sodium Chloride maintains the osmotic balance of the medium. Sodium Azide inhibits cytochrome oxidase in gram-negative bacteria.

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

Formula

Azide Dextrose Broth

Formula Per Liter

Bacto Beef Extract	4.5 g
Bacto Tryptose	15 g
Bacto Dextrose	7.5 g
Sodium Chloride	7.5 g
Sodium Azide	0.2 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

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

Storage

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.47% (single strength) and 6.94% (double strength) solution, soluble in distilled or deionized water. Single-strength solution is light to medium amber, clear to very slightly opalescent; double-strength solution is medium to dark amber, clear.
Prepared Medium:	Light to medium amber, clear (single strength).
Reaction of 3.47% Solution at 25°C:	pH 7.2 ± 0.2

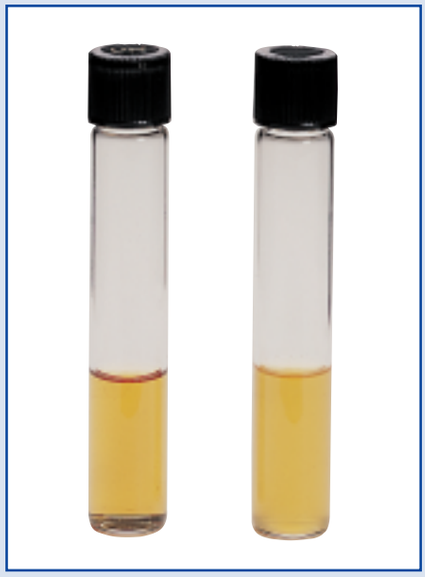
Cultural Response

Prepare Azide Dextrose Broth per label directions. Inoculate 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*	1,000-2,000	inhibited

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

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



Uninoculated tube

Enterococcus faecalis ATCC® 29212

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

Azide Dextrose Broth

Materials Required but not Provided

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

Method of Preparation

1. Suspend 34.7 grams in 1 liter distilled or deionized water. Rehydrate with proportionally less water when liquid inocula will exceed 1 ml.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Presumptive Test Procedure[5]

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

Results

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

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

Limitations of the Procedure

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

References

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

Packaging

Azide Dextrose Broth	500 g	0387-17

Bacto® B_{12} Assay Medium USP

Intended Use

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

Also Known As

USP is an abbreviation for United States Pharmacopeia.

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.

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

Principles of the Procedure

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

Formula

B_{12} Assay Medium USP

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	15 g
Bacto Dextrose	40 g
Bacto Asparagine	0.2 g
Sodium Acetate	20 g
Ascorbic Acid	4 g
L-Cystine	0.4 g
DL-Tryptophane	0.4 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Xanthine	20 mg
Riboflavin	1 mg
Thiamine Hydrochloride	1 mg
Biotin	10 µg
Niacin	2 mg
p-Aminobenzoic Acid	2 mg
Calcium Pantothenate	1 mg
Pyridoxine Hydrochloride	4 mg
Pyridoxal Hydrochloride	4 mg
Pyridoxamine Hydrochloride	800 µg
Folic Acid	200 µg
Monopotassium Phosphate	1 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg
Sorbitan Monooleate Complex	2 g

Final pH 6.0 ± 0.1 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light to light beige, homogeneous, with a tendency to clump.
Solution:	4.25% (single strength) or 8.5% (double strength) solution, soluble in distilled or deionized water on boiling for 2-3 minutes. Light amber, clear, may have a slight precipitate (single strength).
Prepared Medium:	(Single strength) very light to light amber, clear, may have a slight precipitate.
Reaction of 4.25% Solution at 25°C:	pH 6.0 ± 0.1

Cultural Response

Prepare B_{12} Assay Medium USP per label directions. Prepare a standard curve using USP Cyanocobalamin Reference Standard at levels of 0.0 to 0.25 ng per 10 ml. The medium supports the growth of *L. delbrueckii* subsp. *lactis* ATCC® 7830 when supplemented with cyanocobalamin (vitamin B_{12}).

Precautions

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

Storage

Store 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

B_{12} Assay Medium USP

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus delbrueckii* subsp. *lactis* ATCC® 7830
Lactobacilli Agar AOAC or B_{12} Culture Agar USP
Lactobacilli Broth AOAC or B_{12} Inoculum Broth USP
Sterile 0.85% saline
Distilled or deionized water
Spectophotometer or nephelometer
B12 Culture Agar USP
B12 Inoculum Broth USP
Cyanocobalamin USP (vitamin B_{12})

Method of Preparation

1. Suspend 8.5 grams in 100 ml distilled or deionized water.
2. Heat to boiling for 2-3 minutes to dissolve completely.
3. Distribute 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assay, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Follow assay procedures as outlined in USP[1] or AOAC.[2] Use levels of B_{12} in the preparation of the standard curve according to these

references. It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. Generally satisfactory results are obtained with B_{12} at the following levels: 0.0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 and 0.25 ng per assay tube (10 ml).

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

Results

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

Limitations of the Procedure

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

References

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

Packaging

B_{12} Assay Medium USP	100 g	0457-15

Bacto® B_{12} Culture Agar USP
Bacto B_{12} Inoculum Broth USP

Intended Use

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

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

Also Known As

USP is an abbreviation for United States Pharmacopeia.

Summary and Explanation

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

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

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

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

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

Principles of the Procedure

Proteose Peptone No. 3 provides the nitrogen and amino acids in B_{12} Culture Agar USP and B_{12} Inoculum Broth USP. Yeast Extract is the vitamin source in the formulas. Tomato Juice is added to create the proper acidic environment. Dextrose is the carbon source, and Sorbitan Monooleate Complex acts an emulsifier. Potassium Phosphate Dibasic acts as the buffering agent in B_{12} Inoculum Broth USP, and Monopotassium Phosphate is the buffering agent in B_{12} Culture Agar USP. Bacto Agar is the solidifying agent in B_{12} Culture Agar USP.

Formula

B_{12} Culture Agar USP

Formula Per Liter

Tomato Juice	100 ml
Bacto Proteose Peptone No. 3	7.5 g
Bacto Yeast Extract	7.5 g
Bacto Dextrose	10 g
Monopotassium Phosphate	2 g
Sorbitan Monooleate Complex	1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.1 at 25°C

B_{12} Inoculum Broth USP

Formula Per Liter

Tomato Juice	100 ml
Bacto Proteose Peptone No. 3	7.5 g
Bacto Yeast Extract	7.5 g
Bacto Dextrose	10 g
Sorbitan Monooleate Complex	0.1 g
Potassium Phosphate Dibasic	2 g

Final pH 6.8 ± 0.1 at 25°C

Precautions

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

User Quality Control

Identity Specifications

B_{12} Culture Agar USP

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

B_{12} Inoculum Broth USP

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

Cultural Response

B_{12} Culture Agar USP or B_{12} Inoculum Broth USP

Prepare B_{12} Culture Agar USP or B_{12} Inoculum Broth USP per label directions. Inoculate medium with test organism and incubate at 35 ± 2°C for 16-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus delbrueckii subsp. *lactis*	7830	300-1,000	good

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

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.

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

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

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Distilled or deionized water
Inoculating needle

Method of Preparation

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

   B_{12} Culture Agar USP 47 grams

   B_{12} Inoculum Broth USP 32 grams
2. Boil to dissolve completely. (B_{12} Culture Agar)
3. Dispense 10 ml amounts into tubes.
4. Autoclave at 121°C for 15 minutes.
5. Allow tubes of B_{12} Culture Agar to cool in an upright position.

Stock Culture

1. Prepare stock cultures in triplicate in sterile B_{12} Culture Agar USP.
2. Inoculate the tubes using a straight wire inoculating needle.
3. Incubate cultures for 16-24 hours at any temperature between 30-40°C, but held constant within ± 0.5°C.
4. Store at 2-8°C.
5. Before using a fresh culture for assay, make no fewer than 10 successive transfers of the culture in a 2 week period.
6. Prepare stab cultures at least three times each week and do not use a culture for preparing assay inoculum if over 4 days old.

Inoculum

Prepare inoculum as described in USP.[1]

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

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

Results

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

Limitations of the Procedure

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

References

1. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.
2. **Mickle, and Breed**. 1925. Technical Bulletin 110, NY State Agriculture Ex. Station.
3. **Kulp, J. W. L., and V. White.** 1932. Modified medium for plating *Lactobacillus acidophilus*. Science **76**:17.

Packaging

B_{12} Culture Agar USP	100 g	0541-15*
B_{12} Inoculum Broth USP	100 g	0542-15*

*Store at 2-8°C

Bacto® BAGG Broth

Intended Use

Bacto BAGG Broth is used for presumptively identifying and confirming fecal streptococci.

Also Known As

Buffered Azide Glucose Glycerol Medium

Summary and Explanation

In developing Buffered Azide Glucose Glycerol (BAGG) Medium, Hajna[1] modified the formula of SF Broth as specified by Hajna and Perry.[2] Hajna found that adding glycerol to SF Medium enhanced dextrose fermentation by *Enterococcus faecalis*. Decreasing the concentration of brom cresol purple allowed for easier detection of a color change within 24 hours. The BAGG Broth formulation made the original SF Medium more useful in testing for fecal contamination of water and other materials.

User Quality Control

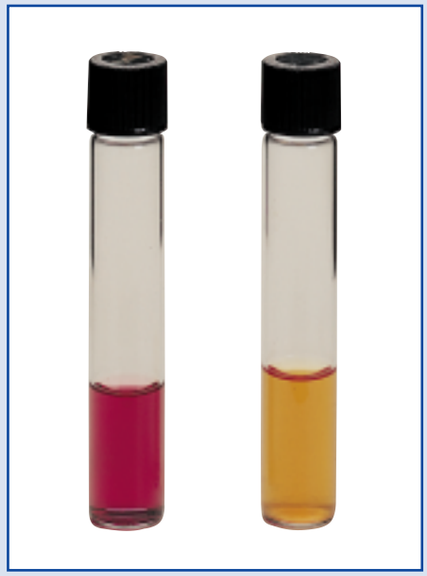
Identity Specifications

Dehydrated Appearance:	Light beige with a slight green tint, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water containing 0.5% glycerol. Solution is purple, clear.
Prepared Tubes:	Purple, clear.
Reaction of 3.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare BAGG Broth per label directions. Inoculate tubes in duplicate and incubate at 35 ± 2°C and 45 ± 1.0°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Enterococcus faecalis	19433*	100-1,000	good	+ (yellow)
Enterococcus faecium	27270	100-1,000	good	+ (yellow)
Escherichia coli	25922*	1,000-2,000	markedly to completely inhibited	–
Streptococcus pyogenes	19615*	1,000-2,000	markedly to completely inhibited	–



Uninoculated tube — *Enterococcus faecalis* ATCC® 29212

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

BAGG Broth contains Tryptose as a source for carbon, nitrogen, vitamins and minerals. Dextrose is a fermentable carbohydrate. Sodium Chloride maintains the osmotic balance of the medium. Sodium Azide inhibits gram-negative bacteria. Brom Cresol Purple is a pH indicator.

Enterococci grow in the presence of azide and ferment glucose, producing an acid pH that changes the color of the medium.

Formula

BAGG Broth

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g
Sodium Azide	0.5 g
Bacto Brom Cresol Purple	0.015 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

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

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

BAGG Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Glycerol
Tubes with closures
Autoclave
Incubators (35 ± 2°C, 45 ± 1°C)

Method of Preparation

1. Dissolve 36 grams in 1 liter distilled or deionized water containing 5 ml glycerol. Rehydrate with proportionally less water when liquid inocula will exceed 1 ml.
2. Dispense into tubes with closures.
3. Autoclave at 114 -118°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[1-3]

1. Inoculate duplicate tubes with sample. Use single-strength medium for inocula of 1 ml or less. Use double-strength medium for inocula of 10 ml.
2. Incubate one set of tubes at 35 ± 2°C for 18-48 hours. Incubate the second set at 45 ± 1°C for 18-48 hours.
3. Read tubes for growth and acid production.

Results[1-3]

1. A positive test is indicated by the production of a yellow color (acid) throughout the medium. This result is presumptive evidence of the presence of fecal streptococci. Further testing must be performed to confirm this result. Consult appropriate references for further identification of *Enterococcus*.[3]
2. A negative result is indicated by no change in the medium (purple color).

Limitations of the Procedure

1. The concentration of the medium must be adjusted to the inoculum size. Refer to discussion in Test Procedure.

References

1. **Hajna, A. A.** 1951. A buffered azide glucose-glycerol broth for presumptive and confirmative tests for fecal streptococci. Pub. Health Lab. **9**:80-81.
2. **Hajna, A. A., and C. A. Perry.** 1943. A comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am. J. Pub. Health **33**:550-556.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

BAGG Broth	500 g	0442-17

Bacto® BG Sulfa Agar · Bacto SBG Enrichment
Bacto SBG Sulfa Enrichment

Intended Use

Bacto BG Sulfa Agar is used for isolating *Salmonella*.

Bacto SBG Enrichment and Bacto SBG Sulfa Enrichment is used for enriching *Salmonella* prior to isolation procedures.

Also Known As

BG is an abbreviation for Brilliant Green and SBG is an abbreviation for Selenite Brilliant Green.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness.[1] The illness results from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many of these cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. Various poultry products are routinely monitored for *Salmonella* before their distribution for human consumption, but in many instances, contaminated food samples elude detection.

BG Sulfa Agar is a highly selective medium. Osborne and Stokes[2] added 0.1% sodium sulfapyridine to Brilliant Green Agar to enhance the selective properties of this medium for *Salmonella*. This formula is recommended as a selective isolation medium for *Salmonella* following enrichment. It is also recommended for direct inoculation with primary specimens for *Salmonella* isolation.

For food testing, BG Sulfa Agar has been used for detection of *Salmonella* in low and high moisture foods.[3,4] It has also been used for detecting *Salmonella* in feeds and feed ingredients.[5] This medium is recommended when testing foods for *Salmonella* following USDA guidelines.[6,7]

SBG Enrichment and SBG Sulfa Enrichment are prepared according to the formulas described by Stokes and Osborne.[8] The researchers found that whole egg and egg yolk reduced the selective properties of selenite brilliant green enrichment.[2] They also found that the addition of sulfapyridine restored these selective properties.[2]

SBG Enrichment and SBG Sulfa Enrichment are selective enrichments for the isolation of *Salmonella* species, especially from egg products. The shell and the contents of the egg at the time of oviposition are generally sterile or harbor very few microorganisms.[9,10,11] Contamination of the shell occurs afterwards from nesting material, floor litter, and avian fecal matter.[12] Salmonellae are of most concern in egg products.[12]

User Quality Control

Identity Specifications

BG Sulfa Agar

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	5.9% solution, soluble in distilled or deionized water on boiling. Solution is very dark amber, very slightly to slightly opalescent.
Prepared Plates:	Dark reddish-amber, slightly opalescent.
Reaction of 5.9% Solution at 25°C:	pH 6.9 ± 0.2

SBG Enrichment

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.37% solution, soluble in distilled or deionized water; green, opalescent with slight precipitate.
Prepared Medium:	Green, opalescent without significant precipitation.
Reaction of 2.37% Solution at 25°C:	pH 7.2 ± 0.2

SBG Sulfa Enrichment

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.42% solution, soluble in distilled or deionized water; green, opalescent without significant precipitation.
Prepared Medium:	Green, opalescent without significant precipitation.
Reaction of 2.42% Solution at 25°C:	pH 7.2 ± 0.2

continued on following page

Principles of the Procedure

In BG Sulfa Agar, Proteose Peptone and Yeast Extract provide nitrogen, vitamins and minerals. Lactose and Sucrose are the sources of carbohydrates in the medium. Brilliant Green and Sodium Pyridine are complementary in inhibiting gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Phenol Red is the pH indicator that turns the medium a yellow color with the formation of acid when lactose and/or sucrose is fermented. Bacto Agar is a solidifying agent.

Bacto Peptone provides the nitrogen, minerals and amino acids in SBG Enrichment and SBG Sulfa Enrichment. Yeast Extract is the vitamin source. D-Mannitol is the carbon source to stimulate organism growth. The phosphates acts as buffers in the enrichments. Sodium Taurocholate, Sodium Selenite and Brilliant Green are the selective agents. The selective agents are used to inhibit gram positive organisms and enteric bacteria other than *Salmonella*. Sodium Sulfapyridine is added in SBG Sulfa Enrichment to increase selectivity.

Formula

BG Sulfa Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Proteose Peptone No. 3	10 g
Bacto Lactose	10 g
Bacto Saccharose	10 g
Sodium Sulfapyridine	1 g
Sodium Chloride	5 g
Bacto Agar	20 g
Brilliant Green	0.0125 g
Bacto Phenol Red	0.08 g

Final pH 6.9 ± 0.2 at 25°C

SBG Enrichment

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Peptone	5 g
Bacto D-Mannitol	5 g
Sodium Taurocholate	1 g
Sodium Selenite	4 g
Dipotassium Phosphate	2.65 g
Monopotassium Phosphate	1.02 g
Bacto Brilliant Green	0.005 g

Final pH 7.2 ± 0.2 at 25°C

SBG Sulfa Enrichment

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Peptone	5 g
Bacto D-Mannitol	5 g
Sodium Taurocholate	1 g
Sodium Sulfapyridine	0.5 g
Sodium Selenite	4 g
Dipotassium Phosphate	2.65 g
Monopotassium Phosphate	1.02 g
Bacto Brilliant Green	0.005 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. **BG Sulfa Agar**
   For Laboratory Use.

   SBG Enrichment, SBG Sulfa Enrichment
   For Laboratory Use.

2. **SBG Enrichment**

   VERY TOXIC. VERY TOXIC BY INHALATION AND IF SWALLOWED. DANGER OF CUMULATIVE EFFECTS. (EC) 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): Kidney, Liver, Spleen.

   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.

   SBG Sulfa Enrichment

   VERY TOXIC. VERY TOXIC BY INHALATION AND IF SWALLOWED. DANGER OF CUMULATIVE EFFECTS. (EC)

Cultural Response

BG Sulfa Agar

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR OF COLONIES/MEDIUM
Enterococcus faecalis	29212*	1,000-2,000	none	–/no change
Escherichia coli	25922*	100-1,000	none to poor	yellow-green
Salmonella typhimurium	14028*	100-1,000	good	pink-white/red

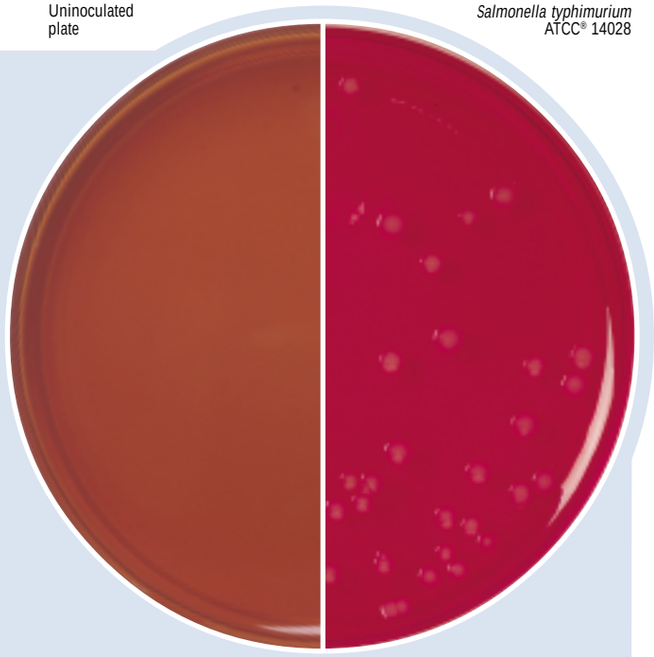
SBG Enrichment and SBG Sulfa Enrichment

Prepare SBG Enrichment and SBG Sulfa Enrichment per label directions. Inoculate tubes with the test organisms. Incubate inoculated medium at 35 ± 2°C for 18-24 hours. After incubation, subculture onto prepared plates of MacConkey Agar.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR ON MACCONKEY
Escherichia coli	25922*	100-1,000	none to poor	pink, if any
Salmonella typhimurium	14028*	100-1,000	good	colorless
Shigella sonnei	9290	100-1,000	poor to fair	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.



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): Kidney, Liver, Spleen.

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 and enrichments below 30°C. The dehydrated products are very hygroscopic. Keep container tightly closed.

Store prepared BG Sulfa Agar 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

BG Sulfa Agar
SBG Enrichment
SBG Sulfa Enrichment

Materials Required But Not Provided

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

Method of Preparation

BG Sulfa Agar

1. Suspend 59 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 will decrease selectivity.
4. Cool to 45-50°C in a waterbath.
5. Dispense into sterile Petri dishes.

SBG Enrichment and SBG Sulfa Enrichment

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

SBG Enrichment	23.7grams/liter
SBG Sulfa Enrichment	24.2 grams/liter

2. Boil gently for 5-10 minutes.
3. Avoid overheating. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

For information about specimen preparation and inoculation of food samples, consult appropriate references.[7,12]

Results

BG Sulfa Agar

The typical *Salmonella* colonies appear as pink-white to red opaque colonies surrounded by a brilliant red medium. The few lactose and/or sucrose fermenting organisms that grow are readily differentiated due to the formation of a yellow-green colony surrounded by an intense yellow-green zone. BG Sulfa Agar is not suitable for the isolation of *S. typhi* or *Shigella*; however, some strains of *S. typhi* may grow forming red colonies.

SBG Enrichment and SBG Sulfa Enrichment

Examine prepared media for growth. Positive tubes should be subcultured onto prepared media for isolation and identification of bacteria.

Limitations of the Procedure

1. On BG Sulfa Agar colonies of *Salmonella* sp. vary from red to pink to white depending on length of incubation and strain.[13]
2. BG Sulfa Agar is normally orange-brown in color; however, on incubation, it turns bright red and returns to normal color at room temperature.[13]
3. *S. typhi* does not grow adequately on BG Sulfa Agar. *Shigella* sp. do not grow on BG Sulfa Agar.[13]
4. Do not sterilize BG Sulfa Agar longer than 15 minutes; longer periods decrease the selectivity of the medium.
5. Since BG Sulfa Agar is highly selective, it is recommended that less selective media, such as MacConkey Agar, be used simultaneously.
6. SBG Enrichment and SBG Sulfa Enrichment should be used in conjunction with selective prepared medium for bacterial identification.

References

1. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Osborn, W. W., and J. L. Stokes.** 1955. A modified selenite brilliant green medium for the isolation of *Salmonella* from egg products. Appl. Microbiol. **3:**295-299.
3. **D'Aoust, J. Y., C. Maishment, D. M. Burgener, D. R. Conley, A. Loit, M. Milling, and U. Purvis.** 1980. Detection of *Salmonella* in refrigerated preenrichment and enrichment broth cultures. J. Food Prot. **43:**343-345.
4. **D'Aoust, J. Y.** 1984. Effective enrichment-plating conditions for detection of *Salmonella* in foods. J. Food Prot. **47:**588-590.
5. **D'Aoust, J. Y., A. Sewell, and A. Boville.** 1983. Rapid cultural methods for detection of *Salmonella* in feeds and feed ingredients. J. Food Prot. **46:**851-855.
6. **Moats, W. A.** 1981. Update on *Salmonella* in foods: selective plating media and other diagnostic media. J. Food Prot. **44:**375-380.

7. **Federal Register.** 1996. Pathogen reduction; hazard analysis and critical point (HACCP) systems; final rule. Fed. Regis. **61:**38917-38925.
8. **Osborn, W. W., and J. L. Stokes.** 1955. Appl. Microbiol. **3**:217.
9. **Brooks, J. and D. J. Taylor.** 1955.Rep. Rd. Invest., Bd. 60, H. M. S. O. London, England.
10. **Forsythe, R. H., J. C. Ayres, and J. L. Radlo.** 1953. Factors affecting the microbiological populations of shell eggs. Food Technol. **7**:49.
11. **Stadelman, W. J., A. I. Ikeme, R. A. Roop, and S. E. Simmons.** 1982. Thermally processed hard-cooked eggs. Poultry Science **61**:388.
12. **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.
13. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

BG Sulfa Agar	500 g	0717-17
SBG Enrichment	500 g	0661-17
SBG Sulfa Enrichment	500 g	0715-17

Bacto® Baird-Parker Agar Base Bacto EY Tellurite Enrichment

Intended Use

Bacto Baird-Parker Agar Base is used with Bacto EY Tellurite Enrichment in isolating and enumerating staphylococci in foods and other materials.

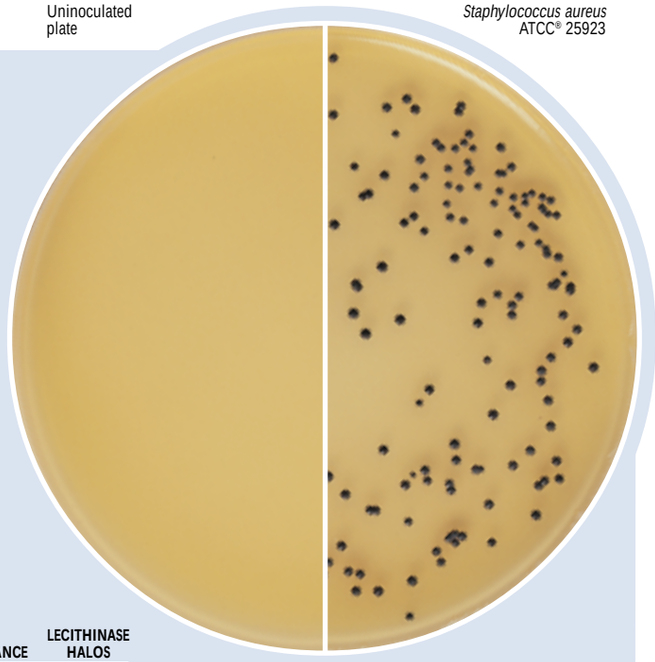
Also Known As

Baird-Parker is also known as Egg Tellurite Glycine Pyruvate Agar (ETGPA) based on its composition.

EY Tellurite Enrichment is also known as Egg Yolk Tellurite Enrichment.

Summary And Explanation

The formulation of Baird-Parker Agar was published in 1962.[1] It is a selective medium for isolation and presumptive identification of coagulase-positive staphylococci.



User Quality Control

Identity Specifications

Baird Parker Agar Base

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

Solution: 6.3% solution, soluble in distilled or deionized water on boiling; light to medium amber, slightly opalescent.

Prepared Medium (Final): Yellow, opalescent.

Reaction of 6.3% Solution at 25°C: pH 6.9 + 0.1

EY Tellurite Enrichment

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

Cultural Response

Baird-Parker Agar Base, EY Tellurite Enrichment

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	LECITHINASE HALOS
Bacillus subtilis	6633	1,000	poor to fair	brown	–
Escherichia coli	25922*	1,000	none	–	N/A
Proteus mirabilis	25933	1,000	good	brown	–
Staphylococcus aureus	25923*	100	good	black	+
Staphylococcus aureus	6538	100	good	black	+
Staphylococcus epidermidis	14990	100	poor to good	black	–

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.

Baird-Parker Agar is widely used and is included in many Standard Methods procedures for testing foods, dairy products and other materials.[2,3,4,5,6] Coagulase-positive staphylococci can grow and reproduce in cosmetic products and these should be tested using standard microbiological methods.[4] In 1995. the American Public Health Association (APHA) published proposed procedures for testing swimming pools for coagulase-positive staphylococci.[7]

Principles of the Procedure

Baird-Parker Agar Base contains Tryptone and Beef Extract as carbon and nitrogen sources for general growth. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Glycine and Sodium Pyruvate stimulate growth of staphylococci. The selectivity of the medium is due to Lithium Chloride and Potassium Tellurite (provided in EY Tellurite Enrichment) which suppress growth of organisms other than staphylococci. The differentiation of coagulase-positive staphylococci depends on the Potassium Tellurite and Egg Yolk (provided in the EY Tellurite Enrichment). Staphylococci that contain lecithinase break down the Egg Yolk and cause clear zones around the colonies. An opaque zone of precipitation may form due to lipase activity. Reduction of Potassium Tellurite, also a characteristic of coagulase-positive staphylococci, causes blackening of the colonies. Bacto Agar is the solidifying agent.

Formula

Baird-Parker Agar Base

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	1 g
Glycine	12 g
Sodium Pyruvate	10 g
Lithium Chloride	5 g
Bacto Agar	20 g

Final pH 6.9 ± 0.1 at 25°C

EY Tellurite Enrichment

Egg yolk emulsion containing Potassium Tellurite.

Precautions

1. For Laboratory Use.
2. **Baird Parker Agar Base**

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, 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 procedures in handling and disposing of infectious materials.

Storage

Store Baird Parker Agar Base below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store EY Tellurite Enrichment at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Baird-Parker Agar Base
EY Tellurite Enrichment

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Petri dishes
Waterbath (45-50°C)
Incubator (35°C)

Method of Preparation

1. Suspend 63 grams Baird-Parker Agar Base in 950 ml 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.
5. Warm EY Tellurite Enrichment to 45-50°C and mix thoroughly to resuspend the precipitate.
6. Aseptically add 50 ml of prewarmed enrichment to the medium. Mix thoroughly.

Specimen Collection and Preparation

Certain foods and other materials may require repair-selective enrichment if injured cells are suspected or selective enrichment if raw food materials or nonprocessed foods containing large numbers of competing microorganisms are being tested.[2] Consult standard references for specific instructions for the type of material being tested.[2,3,4,5]

Test Procedure

1. Prepare dilutions of test samples if indicated by standard procedure.[2,3,4,5]
2. Transfer 1 ml of sample to each of 3 Baird-Parker Agar plates and distribute over the surface using a sterile, bent glass rod.
3. Allow the inoculum to be absorbed by the medium (about 10 minutes) before inverting the plates.
4. Incubate at 35-37°C for 45-48 hours.
5. Examine plates having 20-200 colonies, counting colonies typical of *S. aureus.*

Results

Coagulase-positive staphylococci produce black, shiny, convex colonies with entire margins and clear zones, with or without an opaque zone, around the colonies.

Coagulase-negative staphylococci produce poor or no growth. If growth occurs, colonies are black; clear or opaque zones are rare.

Most other organisms are inhibited or grow poorly. If growth occurs, colonies are light to brown-black with neither clear nor opaque zones.

Limitations of the Procedure

Baird-Parker Agar is selective for coagulase-positive staphylococci but other bacteria may grow. Microscopic examination and biochemical tests will differentiate coagulase-positive staphylococci from other microorganisms.

References

1. **Baird-Parker, A. C.** 1962. An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bacteriol. **25**:12-19.
2. **Lancette, G. A., and S. R. Tatini**. 1992. *Staphylococcus aureus*, p. 533-550. *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. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall, (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Andrews, W. H.** 1995. Microbial Methods, p. 1-119. Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
6. **United States Pharmacopeial Convention.** 1995. The United States Pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Recreational waters, p. 9.26-9.27. *In* Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Baird-Parker Agar Base	100 g	0768-15
	500 g	0768-17
	2 kg	0768-07
	10 kg	0768-08
EY Tellurite Enrichment	6 x 100 ml	0779-73

Bacto® Beef Extract
Bacto Beef Extract, Desiccated

Intended Use

Bacto Beef Extract and Bacto Beef Extract, Desiccated are used in preparing microbiological culture media.

Summary and Explanation

Beef Extract is prepared and standardized for use in microbiological culture media, where it is generally used to replace infusions of meat. Culture media containing Beef Extract have been recommended for use in the bacteriological examination of water, milk and other materials where having media of uniform composition is important.

Beef Extract has been employed by many investigators. Bedell and Lewis[1] used it in their medium for the study of non-sporulating anaerobes of the intestinal tract. Hutner[2] used a medium containing Beef Extract as a stock broth in the study of nutritional needs of streptococci. Beef Extract is the formula of Potato Infusion Agar for the cultivation of *Brucella*. Fletcher Medium Base, Starch Agar, Dextrose Agar, Dextrose Broth and CLED Agar all contain Beef Extract to enhance the growth of bacteria. Antibiotic Assay media specified by US Pharmacopeia[3] includes Beef Extract in the formula. Several media containing Beef Extract are recommended in standard methods for multiple applications.[4,5,6]

In culture media, Beef Extract is usually employed in concentrations of 0.3%. Concentrations may vary slightly according to the requirements of individual formulas, but do not often exceed 0.5%. Beef Extract may be relied upon for biochemical studies, particularly fermentation reactions, because of its independence from fermentable substances that would interfere with the accuracy of such determinations.

Beef Extract, Desiccated, the dried form of Beef Extract, was developed to provide a product for ease of use in handling. Beef Extract is in the paste form. The products are to be used in a one for one substitution, however variations tend to be formulation specific and require actual performance testing.

Principles of the Procedure

Beef Extract and Beef Extract, Desiccated are replacements for infusion of meat. Beef Extract and Beef Extract, Desiccated provide nitrogen, vitamins, amino acids and carbon in several formulations of microbiological culture media.

Typical Analysis

	BEEF EXTRACT	BEEF EXTRACT, DESICCATED
Physical Characteristics		
Ash (%)	24.1	10.2
Clarity, 1% Soln (NTU)	116.8	1.7
Filterability (g/cm^2)	0.1	0.6
Loss on Drying (%)	77.2	2.5
pH, 1% Soln	5.4	6.9
Carbohydrate (%)		
Total	0.2	<0.1
Nitrogen Content (%)		
Total Nitrogen	11.2	14.0
Amino Nitrogen	3.8	2.2
AN/TN	33.8	15.7

	BEEF EXTRACT	BEEF EXTRACT, DESICCATED
Amino Acids (%)		
Alanine	2.54	8.96
Arginine	1.39	5.66
Aspartic Acid	1.67	4.30
Cystine	0.18	0.17
Glutamic Acid	6.01	12.55
Glycine	4.14	16.25
Histidine	4.94	2.50
Isoleucine	0.53	1.45
Leucine	1.00	3.63
Lysine	1.45	3.27
Methionine	0.30	1.08
Phenylalanine	<0.01	2.00
Proline	2.16	9.58
Serine	0.90	2.10
Threonine	0.67	1.42
Tryptophan	0.05	0.32
Tyrosine	1.99	1.03
Valine	0.86	2.62

User Quality Control

Identity Specifications

Beef Extract

Dehydrated Appearance: Medium to dark brown paste.

Solution: 0.3% solution - soluble in distilled or deionized water upon warming. Light to medium amber in color, clear, no precipitate.

Reaction of 0.3% Solution at 25°C: pH 6.9 ± 0.2

Beef Extract, Desiccated

Dehydrated Appearance: Medium to dark brown, free-flowing, homogeneous powder.

Solution: 0.3% solution - soluble in distilled or deionized water at a 0.3% concentration. 0.3% solution is light to medium amber in color, clear without a precipitate.

Reaction of 0.3% Solution at 25°C: pH 6.6-7.4

Cultural Response

Beef Extract

Prepare a sterile solution of 0.3% Beef Extract or Beef Extract, Desiccated, and 0.5% Bacto Peptone. Adjust the pH to 6.9-7.1. Inoculate tubes with the test organisms, incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Salmonella typhimurium	14028*	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 Bactrol Disks Technical Information.

	BEEF EXTRACT	BEEF EXTRACT, DESICCATED
Inorganics (%)		
Calcium	0.068	0.018
Chloride	1.284	1.576
Cobalt	<0.001	<0.001
Copper	<0.001	0.001
Iron	<0.001	0.001
Lead	<0.001	<0.001
Magnesium	0.239	0.022
Manganese	<0.001	<0.001
Phosphate	5.458	0.345
Potassium	5.477	1.994
Sodium	2.315	2.774
Sulfate	0.629	0.829
Sulfur	0.707	0.661
Tin	<0.001	<0.001
Zinc	<0.001	0.002
Vitamins (µg/g)		
Biotin	0.1	0.1
Choline (as Choline Chloride)	1171.5	1300.0
Cyanocobalamin	0.5	<0.1
Folic Acid	3.3	0.6
Inositol	4113.2	2100.0
Nicotinic Acid	774.7	138.1
PABA	20.0	40.5
Pantothenic Acid	91.0	8.7
Pyridoxine	7.3	2.8
Riboflavin	0.4	<0.1
Thiamine	<0.1	<0.1
Thymidine	1093.4	111.3
Biological Testing (CFU/g)		
Coliform	negative	negative
Salmonella	negative	negative
Spore Count	299	585
Standard Plate Count	117	690
Thermophile Count	33	28

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Beef Extract
Beef Extract, Desiccated

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Beef Extract or Beef Extract, Desiccated in the formula of the medium being prepared. Add Beef Extract or Beef Extract, Desiccated 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 Beef Extract or Beef Extract, Desiccated.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on prepared medium.
2. Formula allowances may be required due to the lower sodium chloride concentration of Beef Extract, Desiccated.

References

1. **Bedell and Lewis.** 1938. J. Bacteriol. **36**:567.
2. **Hutner.** 1938. J. Bacteriol. **35**:429.
3. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd. Ed. The United States Pharmacopeial Convention. Rockville, MD.
4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Beef Extract	500 g	0126-17
Beef Extract, Desiccated	500 g	0115-17
	10 kg	0115-08

Bacto® BiGGY Agar

Intended Use

Bacto BiGGY Agar is used for isolating and differentiating *Candida* spp.

Also Known As

BiGGY Agar is an abbreviation for Bismuth Glucose Glycine Yeast Agar. BiGGY Agar is also referred to as Nickerson Agar and Nickerson Candida Elective Agar.

Summary and Explanation

BiGGY Agar is a modification of the formula described by Nickerson.[1,2] This medium was developed while studying sulfite reduction of *Candida* species. Nickerson described BiGGY Agar as a selective and differential medium for the isolation of *Candida albicans*. *C. albicans* can be differentiated from other *Candida* species based on colony morphology.

Candidiasis is the most frequently encountered opportunistic fungal infection.[3] It is caused by a variety of species of *Candida*, with *Candida albicans* being the most frequent etiological agent, followed by *Candida tropicalis* and *Candida (Torulopsis) glabrata*.[3] *Candida* species can be present in clinical specimens as a result of environmental contamination, colonization or actual disease process.[4]

Principles of the Procedure

Yeast Extract provides the nitrogen, vitamins and amino acids in BiGGY Agar. Glycine is used to stimulate growth. Dextrose is the carbon source. *Candida* species reduce bismuth sulfite, and colonies become brown to black in color. Bismuth Sulfite Indicator is also used as a selective agent against bacteria, often present as normal flora. Bacto Agar is used as the solidifying agent.

Formula

BiGGY Agar

Formula Per Liter

Bacto Yeast Extract	1 g
Glycine	10 g
Bacto Dextrose	10 g
Bismuth Sulfite Indicator	8 g
Bacto Agar	20 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

BiGGY Agar

Materials Required But Not Provided

Glassware
Incubator (30°C)
Waterbath (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 49 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Avoid overheating. DO NOT AUTOCLAVE.
3. Evenly disperse the flocculent precipitate when dispensing.

Specimen Collection and Preparation

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

Test Procedure

For a complete discussion on the isolation and identification of yeast species refer to the procedures described in appropriate references.[3,4]

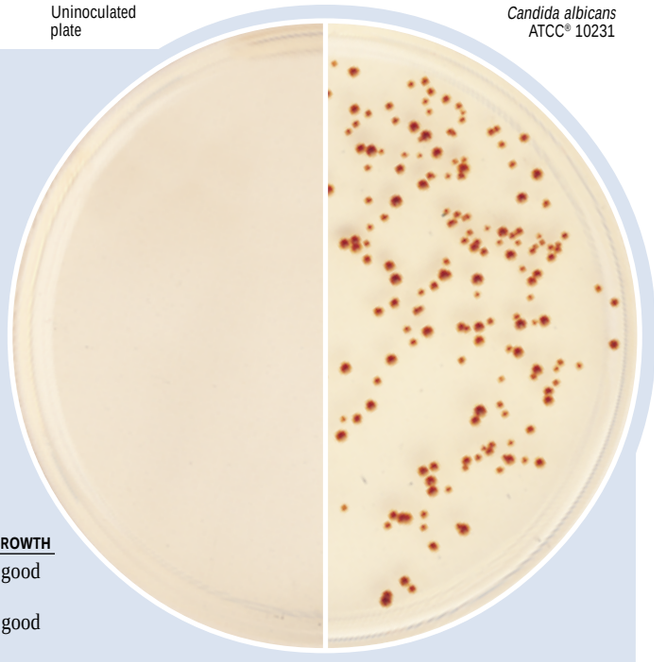
Results

Colony morphology according to Nickerson[2] after 48 hours of incubation on BiGGY Agar:

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

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. Pigmented bacterial and yeast-like fungi are usually inhibited on BiGGY Agar. They can be differentiated by microscopic examination, if necessary. Dermatophytes and molds seldom appear and are easily recognized by development of aerial mycelia.[5]
3. Further growth characteristic and biochemical tests are needed to differentiate yeasts, particularly identification of *Candida* species.[5]



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.9% solution, soluble upon boiling in distilled or deionized water. Solution is very light to light amber, opalescent with a flocculent dispersable precipitate.
Prepared Medium:	Very light to light amber, opalescent with a flocculent precipitate.
Reaction of 4.9% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	COLONY DESCRIPTION	GROWTH
Candida albicans	10231	100-1,000	brown to black, no diffusion into medium, no sheen	good
Candida kefyr	4135	100-1,000	reddish brown, flat colonies, no diffusion	good
Candida tropicalis	750	100-1,000	brown to black, sheen, black diffusion into medium	good
Escherichia coli	25922*	1,000-2,000	–	markedly 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.

4. It is recommended that BiGGY Agar be prepared fresh, just prior to use.[1,2]
5. Do not use slants because the reactions are unsatisfactory.[1,2]

References

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5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 65-68. Williams & Wilkins, Baltimore, MD.

Packaging

BiGGY Agar	100 g	0635-15
	500 g	0635-17

Bacto® Bile Esculin Agar Base
Bacto Bile Esculin Agar

Intended Use

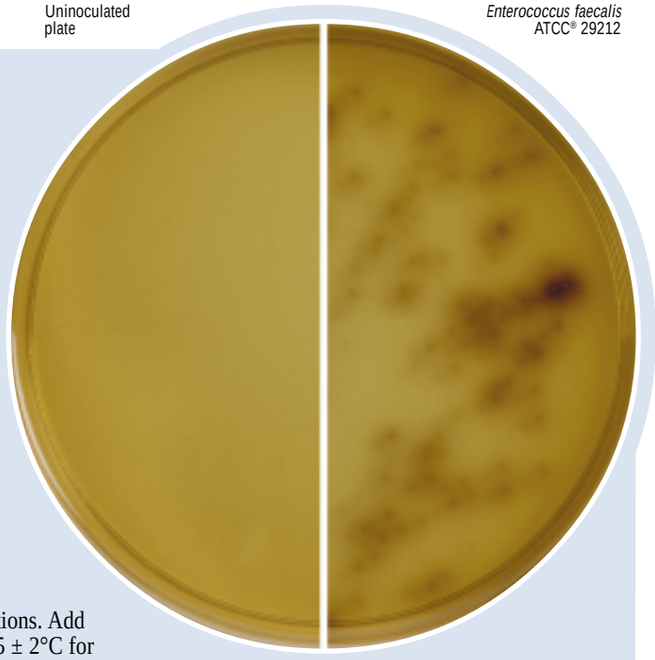
Bacto Bile Esculin Agar Base (with added esculin) and Bacto Bile Esculin Agar are differential media used for isolating and presumptively identifying group D streptococci.

Also Known As

Bile Esculin Agar is also known as Bile Esculin Medium (BEM). The spelling, aesculin, is often seen in literature.

Summary and Explanation

Bile Esculin Agar Base and Bile Esculin Agar are prepared according to the formulation described by Swan[1] and further evaluated by



User Quality Control

Identity Specifications

Dehydrated Appearance:	Greenish, light to medium beige, homogeneous, free-flowing.
Solution:	6.3% solution Bile Esculin Agar Base; 6.4% solution Bile Esculin Agar: soluble in distilled or deionized water on boiling. Solutions are medium to dark amber, slightly opalescent; media with esculin have a bluish cast.
Prepared Plates:	Greenish to medium amber, slightly opalescent; media with esculin have a bluish cast.
Reaction of Solution at 25°C:	6.3% solution Bile Esculin Agar Base; 6.4% solution Bile Esculin Agar: pH 6.6 ± 0.2

Cultural Response

Prepare Bile Esculin Agar Base or Bile Esculin Agar per label directions. Add 0.1% esculin to Bile Esculin Agar Base. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ESCULIN HYDROLYSIS
Enterococcus faecalis	29212*	100-1,000	good	+
Streptococcus pyogenes	19615*	2,000-10,000	inhibited	–

+ = positive, blackening of medium – = negative, 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.

Facklam and Moody.[2] Rochaix[3] first noted the value of esculin hydrolysis in the identification of enterococci. Meyer and Schönfeld[4] added bile to the esculin medium and demonstrated that 61 of 62 enterococci strains were able to grow and hydrolyze esculin, while the other streptococci could not.

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously considered group D streptococci, in the distinct genus *Enterococcus*.[6] Streptococci with Lancefield group D antigen include the nonhemolytic species *Streptococcus bovis*.[7] The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci.

Swan[1] compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping. He reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction. Facklam and Moody,[2] in a comparative study of tests used to presumptively identify group D streptococci, found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci. Facklam[5] further confirmed the usefulness of Bile Esculin Agar in another study differentiating enterococci/group D streptococci from non-group D streptococci.

Lindell and Quinn[8] showed that the medium is also useful in the differentiation of the *Klebsiella-Enterobacter-Serratia* group from other *Enterobacteriaceae.* Edberg et al.[9] recommended the medium for routine testing of the *Enterobacteriaceae* in order to differentiate *Klebsiella-Enterobacter-Serratia* spp. Bile Esculin Agar is listed in standard procedures for the microbiological examination of food products.[10-13]

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxgall (bile) is used to inhibit gram-positive bacteria other than enterococci. Beef Extract and Bacto Peptone provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Bacto Agar is the solidifying agent.

Formula

Bile Esculin Agar

Formula Per Liter	
Bacto Beef Extract	3 g
Bacto Peptone	5 g
Esculin	1 g
Bacto Oxgall	40 g
Ferric Citrate	0.5 g
Bacto Agar	15 g

Final pH 6.6 ± 0.2 at 25°C

Bile Esculin Agar Base

Formula Per Liter	
Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Oxgall	40 g
Ferric Citrate	0.5 g
Bacto Agar	15 g

Final pH 6.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Bile Esculin Agar Base**:

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable gloves and eye/face protection. Use only in well ventilated areas. 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.

   Bacto Bile Esculin 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. TARGET ORGAN(S): Lungs.

   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

Bile Esculin Agar Base
Bile Esculin Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Esculin (to be added to Bile Esculin Agar Base)
Filter-sterilized horse serum (optional)
Petri dishes
Tubes with closures

Method of Preparation

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

   Bile Esculin Agar Base - 63 grams

   Bile Esculin Agar - 64 grams

2. Heat to boiling to dissolve completely.
3. **Bile Esculin Agar Base**, only: Add 1 gram (or another desired amount) of Esculin and mix thoroughly.
4. Autoclave at 121°C for 15 minutes. Overheating may cause darkening of the media.
5. Cool to 50-55°C.
6. If desired, aseptically add 50 ml of filter-sterilized horse serum. Mix thoroughly.
7. Dispense as desired.

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. The bile esculin test was originally formulated to identify enterococci. However, the properties of growth on 40% bile media and esculin hydrolysis are characteristics shared by most strains of Group D streptococci.[14] The bile esculin test should be used in combination with other tests to make a positive identification. Facklam[14] and Facklam et al.[15] recommend a combination of the bile esculin test and salt tolerance (growth in 6.5% NaCl). *Streptococcus bovis* will give a positive reaction on Bile Esculin Agar, but unlike *Enterococcus* spp., it cannot grow on 6.5% NaCl or at 10°C.[16]
2. Bile Esculin Agar should be considered a differential medium, but with the addition of sodium azide (which inhibits gram-negative bacteria) the medium can be made more selective (see Bile Esculin Azide Agar).
3. Occasional viridans strains will be positive on Bile Esculin Agar or will display reactions that are difficult to interpret.[17] Of the viridans group, 5 to 10% may be able to hydrolyze esculin in the presence of bile.[16]
4. Use a light inoculum when testing *Escherichia coli* on Bile Esculin Agar. Wasilauskas[18] suggests that the time required for an isolate to hydrolyze esculin is directly proportional to the size of the inoculum. For a tabulation of those *Enterobacteriaceae* that can hydrolyze esculin, refer to Farmer.[19]

References

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2. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: The bile-esculin test. Appl. Microbiol. **20**:245.
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6. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. **10**:1-19.
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8. **Lindell, S. S., and P. Quinn.** 1975. Use of bile-esculin agar for rapid differentiation of *Enterobacteriaceae.* J. Clin. Microbiol. **1**:440.
9. **Edberg, S. C., S. Pittman, and J. M. Singer.** 1977. Esculin hydrolysis by *Enterobacteriaceae.* J. Clin. Microbiol. **6**:111.
10. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, MD.
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12. **Marshall, R. T. (ed.)** 1992. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
13. **Atlas, R. M.** 1995. Handbook of microbiological media for the examination of food. CRC Press, Boca Raton, FL.
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16. **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.
17. **Ruoff, K. L., S. I. Miller, C. V. Garner, M. J. Ferraro, and S. B. Calderwood.** 1989. Bacteremia with *Streptococcus bovis* and *Streptococcus salivarius:* clinical correlates of more accurate identification of isolates. J. Clin. Microbiol. **27**:305-308.
18. **Wasilauskas, B. L.** 1971. Preliminary observations on the rapid differentiation of the *Klebsiella-Enterobacter-Serratia* group on bile-esculin agar. Appl. Microbiol. **21**:162.
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Packaging

Bile Esculin Agar Base	500 g	0878-17
Bile Esculin Agar	100 g	0879-15
	500 g	0879-17
Esculin	10 g	0158-12

Bacto® Bile Esculin Azide Agar

Intended Use

Bacto Bile Esculin Azide Agar is used for isolating, differentiating and presumptively identifying group D streptococci.

Also Known As

Bile Esculin Azide (BEA) Agar conforms with Selective Enterococcus Medium (SEM) and Pfizer Selective Enterococcus Medium (PSE).

Summary and Explanation

Bile Esculin Azide Agar is a modification of the medium reported by Isenberg[1] and Isenberg, Goldberg and Sampson.[2] The formula modifies Bile Esculin Agar by adding sodium azide and reducing the concentration of bile. The resulting medium is more selective but still provides for rapid growth and efficient recovery of group D streptococci. Enterococcal streptococci were previously grouped in the genus *Streptococcus* with the Lancefield group D antigen. Molecular taxonomic studies have shown that enterococci were sufficiently different from other members of the genus *Streptococcus* to warrant the separate genus *Enterococcus*.[6] Other streptococci with the group D antigen exist in the genus *Streptococcus*, such as the non-hemolytic species *Streptococcus bovis*.[9]

The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci. Esculin hydrolysis and bile tolerance, as shown by Swan[3] and by Facklam and Moody[4], permit the isolation and identification of group D streptococci in 24 hours. Sabbaj, Sutter and Finegold[5] evaluated selective media for selectivity, sensitivity, detection, and enumeration of presumptive group D streptococci from human feces. Bile Esculin Azide Agar selected for *S. bovis*, displayed earlier distinctive reactions, and eliminated the requirement for special incubation temperatures.

Brodsky and Schiemann[6] evaluated Pfizer Selective Enterococcus Medium (Bile Esculin Azide Agar) in the recovery of fecal streptococci from sewage effluent on membrane filters and found the medium to be highly selective for enterococci. Jensen[7] found that Bile Esculin Azide Agar supplemented with vancomycin combines differential and selective properties to rapidly isolate vancomycin-resistant enterococci from heavily contaminated specimens.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to medium beige, free-flowing, homogeneous.
Solution:	5.7% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber with bluish cast, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Medium to dark amber with bluish cast, slightly opalescent.
Reaction of 5.7% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ESCULIN HYDROLYSIS
Enterococcus faecalis	29212*	100-1,000	good	positive, blackening of the medium
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	negative, no 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.

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. Esculetin reacts with ferric ammonium citrate to form a dark brown or black complex. Oxgall (bile) inhibits gram-positive bacteria other than enterococci, while sodium azide inhibits gram-negative bacteria. Tryptone and Proteose Peptone No. 3 provide nitrogen, vitamins and minerals. Yeast Extract provides vitamins and cofactors required for growth, as well as additional sources of nitrogen and carbon. Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent.

Formula

Bile Esculin Azide Agar

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Proteose Peptone No. 3	3 g
Bacto Tryptone	17 g
Bacto Oxgall	10 g
Bacto Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Sodium Chloride	5 g
Sodium Azide	0.15 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

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

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Bile Esculin Azide Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Petri dishes
Horse Serum, filter sterilized (optional)

Method of Preparation

1. Suspend 57 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Overheating may cause darkening of the medium.
4. If desired, aseptically add 50 ml of filter-sterilized horse serum. Mix thoroughly.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For isolation of group D streptococci, inoculate the sample onto a small area of one quadrant of a Bile Esculin Azide Agar plate and streak for isolation. This will permit development of discrete colonies. Incubate at 35°C for 18-24 hours. Examine for colonies having the characteristic morphology of group D streptococci.

Results

Group D streptococci grow readily on this medium and hydrolyze esculin, resulting in a dark brown color around the colonies after 18-24 hours incubation.

Limitations of the Procedure

1. *Staphylococcus aureus* and *Staphylococcus epidermidis* may exhibit growth on the medium (less than 1 mm, white-gray colonies), but they will show no action on the esculin.[2]
2. Other than the enterococci, *Listeria monocytogenes* consistently blackens the medium around colonies. After 18-24 hours, there may be a reddish to black-brown zone of hydrolysis surrounding pinpoint *Listeria* colonies. After 48 hours, white-gray pigmented colonies will be seen. *Listeria* do not attain the same degree of esculin hydrolysis displayed by enterococci in this short incubation period.[2]

References

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Packaging

Bile Esculin Azide Agar	100 g	0525-15
	500 g	0525-17
	2 kg	0525-07

Bacto® Biotin Assay Medium

Intended Use

Bacto Biotin Assay Medium is used for determining biotin concentration by the microbiological assay technique.

Summary and Explanation

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

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

Biotin Assay Medium is prepared for use in the microbiological assay of biotin using *Lactobacillus plantarum* ATCC® 8014 as the test organism.

Principles of the Procedure

Biotin Assay Medium is a biotin-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC® 8014. The addition of biotin standard in specified increasing concentrations gives a growth response by this organism that can be measured titrimetrically or turbidimetrically.

Formula

Biotin Assay Medium

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	12	g
Bacto Dextrose	40	g
Sodium Acetate	20	g
L-Cystine	0.2	g
DL-Tryptophane	0.2	g
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Thiamine Hydrochloride	2	mg
Riboflavin	2	mg
Niacin	2	mg
Calcium Pantothenate	2	mg
Pyridoxine Hydrochloride	4	mg
p-Aminobenzoic Acid	200	µg
Dipotassium Phosphate	1	g
Monopotassium Phosphate	1	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20	mg
Ferrous Sulfate	20	mg
Manganese Sulfate	20	mg

Final pH 6.8 ± 0.2 at 25°C

Precautions

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, homogeneous with a tendency to clump.
Solution:	3.75% (single strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. Light amber, clear, may have a slight precipitate.
Prepared Medium:	(Single strength) light amber, clear, may have slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Biotin Assay Medium per label directions. Prepare a standard curve using biotin at levels of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1 ng per 10 ml. The medium supports the growth of *L. plantarum* ATCC® 8014 when prepared in single strength and supplemented with biotin.

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Biotin Assay Medium

Materials Required But Not Provided

Lactobacilli Agar AOAC
Centrifuge
Spectrophotometer
Biotin
Glassware
Autoclave
Sterile tubes
Stock culture of *Lactobacillus plantarum* ATCC® 8014
Sterile 0.85% saline
Distilled or deionized water

Method of Preparation

1. Suspend 7.5 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 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

Stock Cultures

Stock cultures of the test organism, *L. plantarum* ATCC® 8014, are prepared by stab inoculation of Lactobacilli Agar AOAC. After 16-24 hours incubation at 35-37°C, the tubes are stored in the refrigerator. Transfers are made weekly.

Inoculum

Inoculum for assay is prepared by subculturing from a stock culture of *L. plantarum* ATCC® 8014 to 10 ml of single-strength Biotin Assay Medium supplemented with 0.5 ng biotin. After 16-24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are washed three times with 10 ml sterile 0.85% saline. After the third wash, the cells are resuspended in 10 ml sterile 0.85% saline and finally diluted 1:100 with sterile 0.85% saline. One drop of this suspension is used to inoculate each 10 ml assay tube.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using biotin at levels of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1 ng per assay tube (10 ml).

The concentration of biotin required for the preparation of the standard curve may be prepared by dissolving 0.1 gram of d-Biotin or equivalent in 1,000 ml of 25% alcohol solution (100 µg per ml). Dilute the stock solution by adding 2 ml to 98 ml of distilled water. This solution is diluted by adding 1 ml to 999 ml distilled water, giving a solution of 2 ng of biotin per ml. This solution is further diluted by adding 10 ml to 90 ml distilled water, giving a final solution of 0.2 ng of biotin per ml. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 ml of this final solution. Prepare the stock solution fresh daily.

Biotin Assay Medium may be used for both turbidimetric and titrimetric analysis. Before reading, the tubes are refrigerated for 15-30 minutes to stop growth. Turbidimetric readings should be made after 16-20 hours at 35-37°C. Titrimetric determinations are made after 72 hours incubation at 35-37°C. The most effective assay range, using Biotin Assay Medium, has been found to be between 0.1 ng and 1 ng biotin.

For a complete discussion of antibiotic assay methodology, refer to appropriate procedures outlined in the references.[1,2]

Results

Calculations

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

Limitations of the Procedure

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

References

1. **Federal Register.** 1992. Tests and methods of assay of antibiotics and antibiotic-containing drugs. Fed. Regist. **21**:436.100-436.106.
2. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. Biological tests and assay, p. 1690-1696. The United States Pharmacopeial Convention, Rockville, MD.

Packaging

Biotin Assay Medium	100 g	0419-15

Bacto® Bismuth Sulfite Agar

Intended Use

Bacto Bismuth Sulfite Agar is used for isolating *Salmonella* spp, particularly *Salmonella typhi*, from food and clinical specimens.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with nontyphi *Salmonella* often causes mild, self-limiting illness.[1] Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can produce fatal respiratory, hepatic, splenic, and/or neurological damage. These illnesses result from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. United States federal guidelines require various poultry products to be routinely monitored before distribution for human consumption but contaminated food samples often elude monitoring.

Bismuth Sulfite Agar is a modification of the Wilson and Blair[2-4] formula. Wilson[5,6] and Wilson and Blair[2-4] clearly showed the superiority of Bismuth Sulfite medium for isolation of *S. typhi*. Cope and Kasper[7] increased their positive findings of typhoid from 1.2 to 16.8% among food handlers and from 8.4 to 17.5% among contacts with Bismuth Sulfite Agar. Employing this medium in the routine laboratory examination of fecal and urine specimens, these same authors[8] obtained 40% more positive isolations of *S. typhi* than were obtained on Endo medium. Gunther and Tuft,[9] employing various media in a comparative way for the isolation of typhoid from stool and urine specimens, found Bismuth Sulfite Agar most productive. On Bismuth Sulfite Agar, they obtained 38.4% more positives than on Endo Agar, 33% more positives than on Eosin Methylene Blue Agar, and 80% more positives on Bismuth Sulfite Agar than on the Desoxycholate media. These workers found Bismuth Sulfite Agar to be superior to Wilson's original medium. Bismuth Sulfite Agar was stable, sensitive and easier to prepare. Green and Beard,[10] using Bismuth Sulfite Agar, claimed that this medium successfully inhibited sewage organisms. The value of Bismuth Sulfite Agar as a plating medium after enrichment has been demonstrated by Hajna and Perry.[11]

Since these earlier references to the use of Bismuth Sulfite Agar, this medium has been generally accepted as routine for the detection of most *Salmonella*. The value of the medium is demonstrated by the many references to the use of Bismuth Sulfite Agar in scientific publications, laboratory manuals and texts. Bismuth Sulfite Agar is used in microbial limits testing as recommended by the United States Pharmacopeia. In this testing, pharmaceutical articles of all kinds, from raw materials to the finished forms, are evaluated for freedom from *Salmonella* spp.[12]

For food testing, the use of Bismuth Sulfite Agar is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milks, and butter.[1,13-15] The use of Bismuth Sulfite Agar is also recommended for use in testing clinical specimens.[16,17] In addition, Bismuth Sulfite Agar is valuable when investigating outbreaks of *Salmonella* spp., especially *S. typhi*.[18-20]

Bismuth Sulfite Agar is used for the isolation of *S. typhi* and other *Salmonella* from food, feces, urine, sewage and other infectious materials. The typhoid organism grows luxuriantly on the medium, forming characteristic black colonies, while gram-positive bacteria and members of the coliform group are inhibited. This inhibitory action of Bismuth Sulfite Agar toward gram-positive and coliform organisms permits the use of a much larger inoculum than possible with other media employed for similar purposes in the past. The use of larger inocula greatly increases the possibility of recovering the pathogens, especially when they are present in relatively small numbers. Small numbers of organisms may be encountered in the early course of the disease or in the checking of carriers and releases.

Principles of the Procedure

In Bismuth Sulfite Agar, Beef Extract and Bacto Peptone provide nitrogen, vitamins and minerals. Dextrose is an energy source. Disodium phosphate is a buffering agent. Bismuth sulfite indicator and brilliant green are complementary in inhibiting gram-positive bacteria and members of the coliform group, while allowing *Salmonella* to grow luxuriantly. Ferrous sulfate is for H_2S production. When H_2S is present, the iron in the formula is precipitated, giving positive cultures the characteristic brown to black color with metallic sheen. Agar is a solidifying agent.

Formula

Bismuth Sulfite Agar

Formula Per Liter

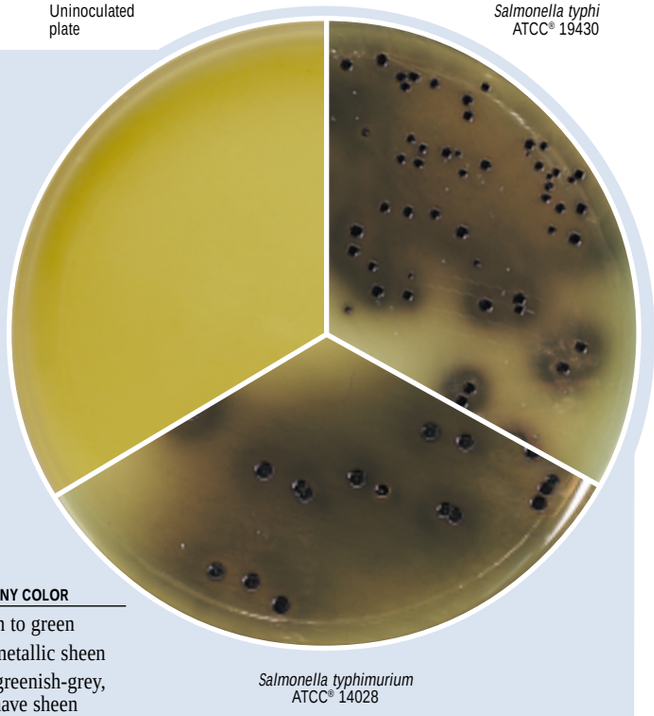
Bacto Beef Extract	5 g
Bacto Peptone	10 g
Bacto Dextrose	5 g
Disodium Phosphate	4 g
Ferrous Sulfate	0.3 g
Bismuth Sulfite Indicator	8 g
Bacto Agar	20 g
Brilliant Green	0.025 g

Final pH 7.7 ± 0.2 at 25°C

Precautions

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

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh 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 to light green, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling. Solution is light green, opaque with a flocculent precipitate that must be dispersed by swirling contents of flask.
Prepared Plates:	Light grey-green to medium green, opaque with a flocculent precipitate.
Reaction of 5.2% solution at 25°C:	7.7 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	1,000-2,000	partial inhibition	brown to green
Salmonella typhi	19430	100-1,000	good	black w/metallic sheen
Salmonella typhimurium	14028*	100-1,000	good	black or greenish-grey, may have sheen
Enterococcus faecalis	29212*	1,000-2,000	markedly 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.

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

Bismuth Sulfite Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 52 grams in 1 liter distilled or deionized water.
2. Heat to boiling no longer than 1-2 minutes to dissolve. Avoid overheating. DO NOT AUTOCLAVE.
3. Cool to 45-50°C in a waterbath.
4. Gently swirl flask to evenly disperse the flocculent precipitate. Dispense into sterile Petri dishes.

   NOTE: Best results are obtained when the medium is dissolved and used immediately. The melted medium should not be allowed to solidify in flasks and remelted. Current references suggest that the prepared plated medium should be aged for one day before use.[13,21]

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.[1,13-20]
2. Process each specimen, using procedures appropriate for that specimen or sample.[1,13-20]

Test Procedure

For isolation of *Salmonella* spp. from food, samples are enriched and selectively enriched. Streak 10 µl of selective enrichment broth onto Bismuth Sulfite Agar. Incubate plates for 24-48 hours at 35°C. Examine plates for the presence of *Salmonella* spp. Refer to appropriate references for the complete procedure when testing food samples.[1,13-15]

For isolation of *Salmonella* spp. from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the Bismuth Sulfite Agar plate and streak for isolation. This will permit the development of discrete colonies. Incubate plates at 35°C. Examine at 24 hours and again at 48 hours for colonies resembling *Salmonella* spp.

For additional information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[16-20]

Results

The typical discrete *S. typhi* surface colony is black and surrounded by a black or brownish-black zone which may be several times the size of the colony. By reflected light, preferably daylight, this zone exhibits a distinctly characteristic metallic sheen. Plates heavily seeded with *S. typhi* may not show this reaction except near the margin of the mass inoculation. In these heavy growth areas, this organism frequently appears as small light green colonies. This fact emphasizes the importance of inoculating plates so that some areas are sparsely populated with discrete *S. typhi* colonies. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.

Generally, *Shigella* spp. other than *S. flexneri* and *S. sonnei* are inhibited. *Shigella flexneri* and *Shigella sonnei* strains that do grow on this medium produce brown to green, raised colonies with depressed centers and exhibit a crater-like appearance.

E. coli is partially inhibited. Occasionally a strain will be encountered that will grow as small brown or greenish glistening colonies. This color is confined entirely to the colony itself and shows no metallic sheen. A few strains of *Enterobacter aerogenes* may develop on this medium, forming raised, mucoid colonies. *Enterobacter* colonies may exhibit a silvery sheen, appreciably lighter in color than that produced by *S. typhi*. Some members of the coliform group that produce hydrogen sulfide may grow on the medium, giving colonies similar in appearance to *S. typhi*. These coliforms may be readily differentiated because they produce gas from lactose in differential media, for example, Kligler Iron Agar or Triple Sugar Iron Agar. The hydrolysis of urea, demonstrated in Urea Broth or on Urea Agar Base, may be used to identify *Proteus* sp.

To isolate *S. typhi* for agglutination or fermentation studies, pick characteristic black colonies from Bismuth Sulfite Agar and subculture them on MacConkey Agar. The purified colonies from MacConkey Agar may then be picked to differential tube media such as Kligler Iron Agar, Triple Sugar Iron Agar or other satisfactory differential media for partial identification. All cultures that give reactions consistent with *Salmonella* spp. on these media should be confirmed biochemically as *Salmonella* spp. before any serological testing is performed. Agglutination tests may be performed from the fresh growth on the differential tube media or from the growth on nutrient agar slants inoculated from the differential media. The growth on the differential tube media may also be used for inoculating carbohydrate media for fermentation studies.

Limitations of the Procedure

1. It is important to streak for well isolated colonies. In heavy growth areas, *S. typhi* appears light green and may be misinterpreted as negative growth for *S. typhi*.[22]
2. *S. typhi* and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. Brown zones are not produced by other members of the *Enterobacteriaceae*. However, *S. arizonae* is usually inhibited.[22]
3. Colonies on Bismuth Sulfite Agar may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium (e.g., MacConkey Agar).[22]
4. Typical *S. typhi* colonies usually develop within 24 hours; however, all plates should be incubated for a total of 48 hours to allow growth of all typhoid strains.[22]

5. DO NOT AUTOCLAVE. Heating this medium for a period longer than necessary to just dissolve the ingredients destroys its selectivity.

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Packaging

Bismuth Sulfite Agar	100 g	0073-15
	500 g	0073-17
	10 kg	0073-08

Bacto® Blood Agar Base
Bacto Blood Agar Base No. 2

Intended Use

Bacto Blood Agar Base is used for isolating and cultivating a wide variety of microorganisms and, with added blood, for cultivating fastidious microorganisms.

Bacto Blood Agar Base No. 2 is used for isolating and cultivating fastidious microorganisms with or without added blood.

Also Known As

Blood Agar Base is abbreviated as BAB, and may be referred to as Infusion Agar.

Summary and Explanation

Blood agar bases are typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, blood agar bases can be used as general purpose media.

In 1919, Brown[1] experimented with blood agar formulations for the effects of colony formation and hemolysis; the growth of pneumococci was noticeably influenced when the medium contained peptone manufactured by Difco.

Blood Agar Base is a modification of Huntoon's[2] "Hormone" Medium with a slight acidic composition. Norton[3] found the pH of 6.8 to be advantageous in culturing streptococci and pneumococci. Blood Agar Base No. 2 is a nutritionally rich medium for maximum recovery of fastidious microorganisms.

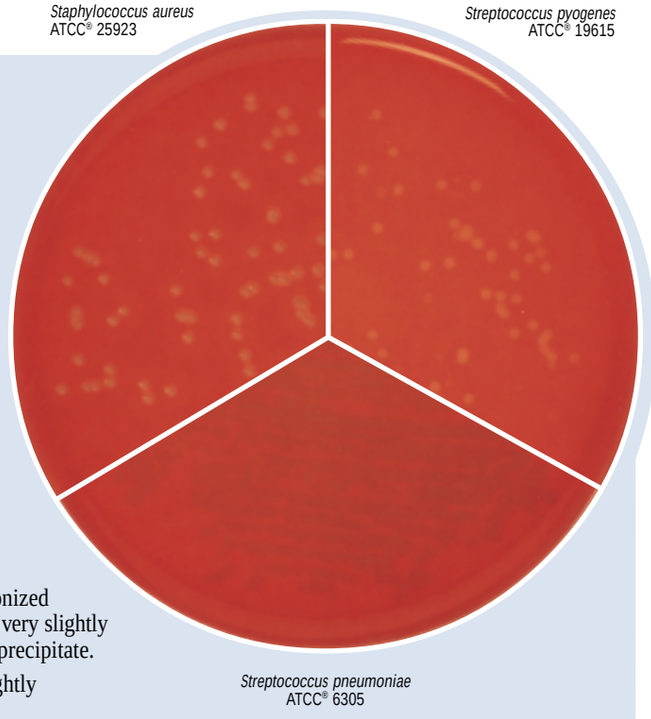
Blood Agar Base media are specified in Standard Methods[4,5,6] for food testing.

Principles of the Procedure

Blood Agar Base formulations have been prepared using specially selected raw materials to support good growth of a wide variety of fastidious microorganisms.

Infusion from Beef Heart and Tryptose provide nitrogen, carbon, amino acids and vitamins in Blood Agar Base. Proteose Peptone No. 3 is the nitrogen source for Blood Agar Base No. 2 while Yeast Extract and Liver Digest provide essential carbon, vitamin, nitrogen and amino acids sources. Both media contain Sodium Chloride to maintain osmotic balance and Bacto Agar as a solidifying agent. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci.[7]

Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood or type of base medium used.[8] Chocolate agar for isolating *Haemophilus* and *Neisseria* species can be prepared from Blood Agar Base No. 2 by supplementing the medium with 10% sterile defibrinated blood (chocolatized).



User Quality Control

Identity Specifications

Blood Agar Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Without blood -light to medium amber, slightly opalescent.
	With 5% sheep blood - cherry red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 6.8 ± 0.2

Blood Agar Base No. 2

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in distilled or deionized water upon boiling; medium to dark amber very slightly to slightly opalescent, without significant precipitate.
Prepared Medium:	Without blood-medium to dark amber, slightly opalescent, without significant precipitate.
	With 5% sheep blood-cherry red, opaque.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Blood Agar Base or Blood Agar Base No. 2 per label directions with and without 5% sterile defibrinated sheep blood. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HEMOLYSIS
Escherichia coli	25922	100-1,000	good	–
Staphylococcus aureus	25923*	100-1,000	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	alpha
Streptococcus pyogenes	19615*	100-1,000	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.

Formula

Blood Agar Base

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Tryptose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Blood Agar Base No. 2

Formula Per Liter

Bacto Proteose Peptone No. 3	15 g
Liver Digest	2.5 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g
Bacto Agar	12 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

Blood Agar Base

Blood Agar Base No. 2

Materials Required But Not Provided

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

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Blood Agar Base - 40 grams;
   Blood Agar Base No. 2 - 39.5 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. To prepare chocolate agar, add 10% sterile defibrinated blood to Blood Agar Base No. 2 at 80°C. Mix well.
6. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

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

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.[8]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.

Results

Examine the medium for growth and hemolytic reactions after 18-24 and 48 hours incubation. Four types of hemolysis on blood agar media can be described:[9]

a. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish discoloration of the medium.
b. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
c. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
d. Alpha-prime hemolysis (ὰ) is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Blood Agar Base media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
2. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
3. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit blood agar and alpha-hemolytic on sheep blood agar.[8]
4. Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[10]
5. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[8] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.

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Packaging

Blood Agar Base	100 g	0045-15
	500 g	0045-17
	2 kg	0045-07
Blood Agar Base No. 2	500 g	0696-17

Bacto® Bordet Gengou Agar Base

Intended Use

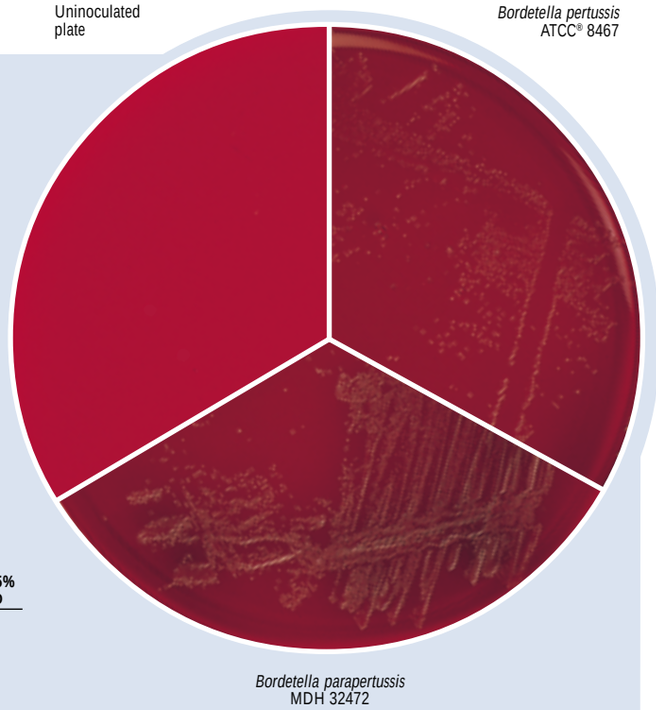
Bacto Bordet Gengou Agar Base is used with added blood for isolating *Bordetella pertussis* and other *Bordetella* species.

Also Known As

Bordet Gengou Agar Base is also referred to as B-G Agar Base and Bordet-Gengou Potato-Glycerol Agar.[1]

Summary and Explanation

Bordet Gengou Agar Base is a modification of the medium originally described by Bordet and Gengou[2] in 1906 for the cultivation of *Haemophilus pertussis*, now *Bordetella pertussis*. The original formula used a base medium consisting of 1% glycerol and potato extract with



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble upon boiling in distilled or deionized water containing 1% glycerol; light to medium amber, opalescent, may have a slight precipitate.
Prepared Medium:	Plain - Light to medium amber, opalescent, may have a precipitate. With 15% blood - Cherry red, opaque.
Reaction of 3.0% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Bordet Gengou Agar Base enriched with 15% sterile defibrinated blood per label directions. Inoculate and incubate at 35 ± 2°C for 48-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH W/O BLOOD	GROWTH W/15% RABBIT BLOOD
Bordetella bronchiseptica	4617	30-300	good	good
Bordetella parapertussis	MDH 32472	30-300	poor to good	good
Bordetella pertussis	8467	30-300	poor to good	good

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

an equal volume of human or rabbit blood. The modified medium is prepared according to the formula recommended by the American Public Health Association.[3] Eldering and Kendrick[4] reported that the addition of 1% proteose peptone or neopeptone increased growth of *B. pertussis*, thereby increasing the yield of vaccine.

The genus *Bordetella* consists of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium*.[5] All *Bordetella* are respiratory pathogens, residing on the mucous membranes of the respiratory tract. *B. pertussis* and *B. parapertussis* are uniquely human pathogens. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder form of the disease.[6] *B. bronchiseptica* is an opportunistic human pathogen associated with both respiratory and non-respiratory infections, often occurring in patients having close contact with animals.[5] *B. bronchiseptica* has not been reported to cause pertussis. There have been no reports of recovery of *B. avium* from humans.[5]

The "cough plate" method for the diagnosis of whooping cough was originally reported by Chievitz and Meyer.[7] This technique is no longer recommended. Nasopharyngeal washings or a nasopharyngeal swab (calcium alginate on a wire handle) should be collected within the first week of paroxysmal coughing.[8]

Principles of the Procedure

Infusion from Potato provides nitrogen, vitamins and amino acids. Glycerol is a carbon source. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent. The addition of blood provides essential growth requirements for *Bordetella* species.

Many factors will inhibit growth of *B. pertussis*, including fatty acids present in nasal secretions or cotton from the collection swab. Starch, present from the Potato Infusion, absorbs fatty acids.

Modified Bordet Gengou medium, enriched with 15-20% blood, yields typical *B. pertussis* growth. The colonies appear small, white, opaque and surrounded by a characteristic zone of hemolysis that is not sharply defined but merges diffusely into the medium. The zone of hemolysis is usually absent if 30% or more blood is added to the medium and cannot be seen on charcoal-containing media.[9] Sterile, defibrinated sheep or rabbit blood can be used in preparing the medium.

Formula

Bordet Gengou Agar Base

Formula Per Liter	
Potato, Infusion from	125 g
Sodium Chloride	5.5 g
Bacto Agar	20 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.

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

Bordet Gengou Agar Base

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 30 grams in 1 liter distilled or deionized water containing 10 grams of glycerol.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C. Aseptically add 15% sterile defibrinated sheep or rabbit blood. Mix well.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation[9]

Specimens should be obtained during the early phases of the disease and prior to the convalescent stage and antimicrobial therapy. The specimen of choice is duplicate nasopharyngeal swabs. Direct plating of the specimen at bedside is recommended; when this is not possible, submerge both swabs into Regan-Lowe transport medium.

Test Procedure[9]

1. Roll one of the swabs over the primary inoculation area of the Bordet Gengou plate and streak for isolation. Return the swab to the transport medium. Incubate the transport medium for 48 hours. Plate the swabs onto a duplicate set of media.
2. Incubate the culture plates at 35°C for 5-7 days in a moist chamber. Increased CO_2 is not recommended. Growth of *B. pertussis* appears in 3-5 days. Other bordetellae species can appear in 1-3 days.
3. Nasopharyngeal specimens may contain staphylococci that produce a diffusible substance inhibitory to *B. pertussis* growth. For these specimens, use a plating medium with methicillin (2.5 µg/ml) or cephalexin (40 µg/ml) and a medium without antimicrobics.
4. Isolates suspected of being *B. pertussis* should be confirmed by using a specific antiserum in either the slide agglutination or fluorescent antibody staining techniques.[10]

Results

For a complete discussion on the isolation and identification of *Bordetella* species refer to the appropriate procedures outlined in the references.

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. Some *Haemophilus* species will grow on *Bordetella* isolation media and may cross-react with *B. pertussis* antisera. It may be prudent to rule out X and V factor dependence.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 86-92. Williams & Wilkins, Baltimore, MD.
2. **Bordet, J., and D. Gengou.** 1906. Le microbe de la coqueluche. Ann. Inst. Pasteur **20**:731.
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6. **Linneman, C. C., and E. B. Pery.** 1977. *Bordetella parapertussis*: recent experience and a review of the literature. Am. J. Dis. Child. **131**:560-563.
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9. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
10. **Koneman, E. W.** 1988. Color atlas and textbook of diagnostic microbiology, 3rd ed. J. B. Lippincott Company, Washington, D.C.

Packaging

Bordet Gengou Agar Base	100 g	0048-15
	500 g	0048-17

Bacto® Bovine Albumin 5%

Intended Use

Bacto Bovine Albumin 5% is used to enrich media for cultivating a large variety of microorganisms and tissue cells.

Also Known As

Bovine Albumin can be abbreviated as BSA.[1]

User Quality Control

Identity Specifications

Bovine Albumin 5%

Appearance:	Light amber, clear to very slightly opalescent.
Sterility Test:	Negative.
Reaction of Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Dubos Broth Base per label directions, substituting Bovine Albumin 5% for Dubos Medium Albumin. Inoculate and incubate at 35 ± 2°C under CO_2 for up to three weeks.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium intracellulare	13950	100-1,000	good
Mycobacterium tuberculosis H37Ra	25177	100-1,000	good
Mycobacterium tuberculosis H37Ra	27294	100-1,000	good

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

Summary and Explanation

Davis and Dubos[2] recommended the use of bovine albumin at a final concentration of 0.5% in liquid media for culturing *Mycobacterium tuberculosis*. In this study, bovine albumin neutralized the toxicity of fatty acids and permitted more luxuriant growth of *M. tuberculosis*.

Ellinghausen and McCullough[3] used bovine albumin fraction V at a final concentration of 1% in liquid, semisolid and solid media for culturing leptospires. Morton et al.[4] demonstrated that 1% bovine albumin stimulated growth of *Mycoplasma* (PPLO).

Bovine Albumin can be added to normally sterile specimens, tissues and body fluids for direct inoculation onto culture media used for isolating mycobacteria. BSA is also used as an enrichment when contaminated specimens are digested.

Bovine Albumin 5%, modified with added sodium chloride and dextrose, is available as Dubos Medium Albumin.

Principles of the Procedure

Bovine Albumin 5% is a filter sterilized solution of Bovine Albumin Fraction V. BSA is suggested as a culture media enrichment because its buffering capacity and detoxifying effect on specimen sediment.[1] Bovine Albumin 5% also increases adhesion of the specimen to solid media.[1]

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.
3. Mycobacterial organisms are BioSafety Level 2 pathogens. The handling of clinical specimen material that is potentially infected with mycobacteria should be performed in a Class I or II biological safety cabinet (BSC).[1]

Storage

Store Bovine Albumin 5% 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

Bovine Albumin 5%

Materials Required But Not Provided

Materials vary depending on the specimen collected and the procedure performed.

Method of Preparation

Refer to the final concentration of Bovine Albumin in the procedure being used to inoculate specimens. A 0.2% solution of Bovine Albumin 5% is recommended for the inoculation of sterile and contaminated specimens when isolating mycobacteria.[1]

Specimen Collection and Preparation

Many different specimen types can be collected for mycobacterial cultures but the majority will be from the respiratory tract.[1] Tissues, body fluids, urine, blood and gastric aspirates can also be tested for the presence of mycobacteria. Refer to the procedures established by laboratory policy or to appropriate references for specific guidelines on specimen collection and processing.

Test Procedure

Sterile Specimens for the Isolation of Mycobacteria[1]

Normally sterile tissues may be ground in 0.2% BSA and inoculated directly in culture media. Concentrate body fluids before inoculation because they normally contain only a small number of mycobacteria. Centrifuge fluids at 3,000 x g and inoculate the sediment onto liquid or solid media. For a complete discussion of the inoculation of sterile specimens, refer to appropriate references.

Contaminated Specimens for the Isolation of Mycobacteria[1]

A concentration of 0.2% Bovine Albumin fraction V can be added to specimen sediment that has been digested and centrifuged by the NALC-NaOH digestion method. Using a separate sterile pipette for each tube, add 1-2 ml of 0.2% BSA, then resuspend the sediment with the pipette or by shaking the tube gently by hand.

Several digestion procedures exist. Consult appropriate references for a complete discussion on all digestion and decontamination methods and other testing procedures.

Results

All media should be examined closely for evidence of growth. Refer to the procedure established by laboratory policy or to appropriate references on typical growth patterns and confirmation tests.

Limitations of the Procedure

1. Bovine Albumin 5% is not recommended for use with Bactec® because BSA may delay detection times.[1]

References

1. **Nolte, F. S., and B. Metchock.** 1995. *Mycobacterium*, p. 400-437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Davis and Dubos.** 1945. J. Bacteriol. **55**:11.
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Packaging

Bovine Albumin 5%	12 x 20 ml	0668-64

Brain Heart Infusion Media

Bacto® Brain Heart Infusion · Bacto Brain Heart Infusion Agar Bacto Clostridium Difficile Antimicrobic Supplement CC Bacto Brain Heart CC Agar · Bacto Brain Heart Infusion w/PAB and Agar · Bacto Brain Heart Infusion w/o Dextrose

Intended Use

Bacto Brain Heart Infusion is used for cultivating fastidious microorganisms, including streptococci, pneumococci and meningococci.

Bacto Brain Heart Infusion Agar is used for cultivating fastidious microorganisms, especially fungi and yeasts, and, with added antibiotics, for isolating fungi.

Bacto Clostridium Difficile Antimicrobic Supplement CC is used with Brain Heart Infusion Agar in preparing Clostridium Difficile Agar.

Bacto Brain Heart CC Agar is used for isolating and cultivating fastidious fungi.

Bacto Brain Heart Infusion w/PAB and Agar is used for cultivating fastidious organisms, particularly from blood containing sulfonamides.

Bacto Brain Heart Infusion w/o Dextrose is used for cultivating fastidious organisms.

Also Known As

Brain Heart Infusion is abbreviated as BHI.

Summary and Explanation

In 1919, Rosenow[1] devised an excellent medium for culturing streptococci by supplementing dextrose broth with brain tissue. Hayden[2] revised Rosenow's procedure by adding crushed marble to the medium and reported favorable growth of organisms from dental pathogens. Brain Heart Infusion is a modification of the media described by Rosenow[1] and Hayden[2] in which infusion from calf brains has replaced the brain tissue and disodium phosphate has replaced the calcium carbonate buffer.

Brain Heart Infusion Agar is used for cultivating a variety of fastidious microorganisms, fungi and yeasts. This medium is used in combination with penicillin and streptomycin. Roseburg, Epps and Clark[3] reported that the isolation and cultivation of *Actinomyces israelii* was enhanced on Brain Heart Infusion with 2% agar compared with 1% dextrose infusion agar. Howell[4] used Brain Heart Infusion with the addition of 2% Bacto Agar and 10% sterile defibrinated horse blood for the cultivation of *Histoplasma capsulatum*.

Brain Heart Infusion Agar can be used with Clostridium Difficile Antimicrobic Supplement CC, a selective supplement containing lyophilized cycloserine and cefoxitin, for the preparation of Clostridium Difficile Agar. The complete medium is based on the formula of Willey and Bartlett[5] and recommended for use in the isolation of *Clostridium difficile* from fecal specimens. *C. difficile* is the major cause of antibiotic-associated diarrhea and pseudomembranous colitis.[6]

Brain Heart CC Agar is prepared with chloramphenicol and cycloheximide (Actidione) according to the formulation of Ajello et al.[7] and McDonough et al.[8] These selective agents restrict growth of bacteria and saprophytic fungi. Brain Heart CC Agar is used in the isolation of fungi that cause systemic disease, such as *Histoplasma capsulatum* and *Blastomyces dermatiditis*.

Brain Heart Infusion media are specified in several standard methods references for food testing.[9,10,11] Standard Methods for the Examination of Water and Wastewater recommends Brain Heart Infusion media in tests for the verification of fecal streptococci.[12]

Brain Heart Infusion is recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for the preparation of inocula used in antimicrobial susceptibility tests.[13]

Brain Heart Infusion w/o Dextrose is a basal medium used with added carbohydrates for fermentation studies.

Modifications of BHI media include:[14]

- Brain Heart Infusion Agar with penicillin (20,000 U) and streptomycin (40 mg) for the selective isolation of pathogenic

User Quality Control

Identity Specifications

Brain Heart Infusion

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water; light to medium amber, clear without significant precipitate.
Prepared Medium:	Light to medium amber, clear without significant precipitate.
Reaction of 3.7% Solution at 25°C	pH 7.4 ± 0.2

Brain Heart Infusion Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling; light to medium amber, slightly opalescent to opalescent with a flocculent precipitate.
Prepared Medium:	Plain - light to medium amber, slightly opalescent with a precipitate. With 5% sheep blood-cherry red, opaque.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Brain Heart CC Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling; medium amber, slightly opalescent without significant precipitate.
Prepared Medium:	Medium amber, slightly opalescent without a precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Brain Heart Infusion w/PAB and Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in distilled or deionized water on boiling; light to medium amber, slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.8% Solution at 25°C:	pH 7.4 ± 0.2

Brain Heart Infusion w/o Dextrose

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water; light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 3.5% Solution at 25°C:	pH 7.4 ± 0.2

Clostridium Difficile Antimicrobic Supplement CC

Lyophilized Appearance:	White, homogeneous cake.
Solution:	Soluble in 5 ml sterile distilled or deionized water. Colorless, clear.
Microbial Limits Test:	Satisfactory (negative).
Reaction of Rehydrated Vial at 25°C:	pH 5.9-6.3

continued on following page

fungi from specimens heavily contaminated with bacteria and saprophytic fungi;

- Brain Heart Infusion with 3% sodium chloride for the isolation of *Vibrio parahaemolyticus*;
- Brain Heart Infusion with agar, yeast extract, sodium chloride, inactivated horse serum and penicillin for the cultivation of fastidious fungi;
- Brain Heart Infusion with casein to support the growth of *Serratia marcescens*;
- Brain Heart Infusion with 0.7% agar to support the growth of staphylococcal species for the production of enterotoxin; and,
- Brain Heart Infusion with rabbit serum and yeast extract for the cultivation of *Mycoplasma equirhinis.*

Principles of the Procedure

Infusion from Beef Heart, Calf Brains and Proteose Peptone provide nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion media. Dextrose is a carbon energy source that facilitates organism growth. Sodium Chloride maintains the osmotic balance of the medium. Disodium Phosphate is a buffering agent. Bacto Agar is a solidifying agent.

The nutritionally rich broth formulation of Brain Heart Infusion supports growth of a variety of microorganisms, as does the medium when supplemented with agar and/or blood. BHI (broth) is often used as a blood culture medium and as a basal medium for metabolic tests, particularly for identifying streptococci.15 BHI with 0.5% Polysorbate 80 can be used for detecting *Mycobacterium avium-intracellulare* complex organisms and *M. tuberculosis* from blood cultures.[15]

Brain Heart Infusion Agar is used in the aminoglycoside and vancomycin screen test for resistant enterococci.[16] BHI Agar with 5-10% sheep blood and chloramphenicol (16 µg/ml) and gentamicin (5 µg/ml) will inhibit the growth of bacteria while allowing growth of dimorphic fungi.[15] This agar can be used as a primary plating medium

User Quality Control cont.

Cultural Response

Brain Heart Infusion (0037)
Brain Heart Infusion w/o Dextrose (0502)
Prepare the selected medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

Brain Heart Infusion Agar (0418)
Prepare medium with and without 5% sheep blood per label directions. Inoculate and incubate *Aspergillus* aerobically at 30 ± 2°C for 18-72 hours; incubate all other organisms aerobically at 35 ± 2°C with 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH PLAIN	GROWTH w/5% SHEEP BLOOD
Aspergillus niger	16404	100-1,000	good	good
Streptococcus pneumoniae	6305	100-1,000	good	good
Streptococcus pyogenes	19615	100-1,000	good	good
Staphylococcus aureus	25923*	100-1,000	good	good

Brain Heart Infusion w/PAB and Agar (0499)
Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours under appropriate atmospheric conditions.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis	25285	100-1,000	good
Neisseria meningitidis	13090*	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

Brain Heart CC Agar (0483)
Prepare medium per label directions. Inoculate and incubate at 25 ± 2°C for up to 7 days

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

Uninoculated tube | *Neisseria meningitidis* ATCC® 13090 | *Streptococcus pyogenes* ATCC® 19615

Brain Heart Infusion

Clostridium Difficile Antimicrobic Supplement CC
Prepare 500 ml Brain Heart Infusion Agar supplemented with 5% sterile sheep blood and 5 ml Clostridium Difficile Antimicrobic Supplement CC. Inoculate and incubate at 35 ± 2°C under anaerobic conditions for 24-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Clostridium difficile	17858	100-1,000	good
Clostridium difficile	9689	100-1,000	good
Clostridium perfringenes	13124*	1,000-2,000	markedly to completely inhibited
Enterococcus faecalis	33186	1,000-2,000	markedly to completely inhibited
Escherichia coli	25922*	1,000-2,000	markedly to completely 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 Disk Technical Information.

for the growth of fungi since it has been shown to yield better recovery than the previously recommended Sabouraud Dextrose Agar.[15] In Brain Heart CC Agar, chloramphenicol is used as a broad-spectrum antibiotic to inhibit a wide range of bacteria; cycloheximide inhibits saprophytic fungi. Sheep blood provides essential growth factors for fastidious fungi.

Clostridium Difficile Agar (Brain Heart Infusion Agar supplemented with 5% sheep blood or 7% horse blood and Clostridium Difficile Antimicrobic Supplement CC) improves the growth and recovery of *C. difficile*. Clostridium Difficile Agar markedly to completely inhibits most aerobic and anaerobic enteric organisms other than *C. difficile*.[5] The final concentration of cycloserine and cefoxitin in Clostridium Difficile Agar is 250 mcg/ml and 10 mcg/ml, respectively.

Brain Heart CC Agar can be supplemented with sheep blood (5-10%) for enrichment and gentamicin (5 mg/l) for additional selectivity.[14] McDonough et al[17] demonstrated that the temperature of incubation affects the sensitivity of some pathogenic fungi to antibiotics. Incubate the medium containing antibiotics at room temperature. The specimen source and the type of fungus suspected will indicate the isolation medium to be used. Both an antimicrobic-containing medium and a non-selective medium should be used on primary isolates with incubation at both 25°C and 37°C.

Brain Heart Infusion w/PAB and Agar contains p-aminobenzoic acid (0.05 g/l) to neutralize sulfonamides in the blood of patients receiving this therapy. This formulation will also inactivate streptomycin in the ratio of 10 ml of medium to 100 units of streptomycin. The addition of 0.1% agar to Brain Heart Infusion w/PAB and Agar provides optimum conditions for aerobic organisms, microaerophiles and obligate anaerobes.

Formula

Brain Heart Infusion

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g

Final pH 7.4 ± 0.2 at 25°C

Brain Heart Infusion Agar

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Brain Heart Infusion w/o Dextrose

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g

Final pH 7.4 ± 0.2 at 25°C

Brain Heart Infusion W/PAB and Agar

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g
Bacto Agar	1 g
p-Aminobenzoic Acid	0.05 g

Final pH 7.4 ± 0.2 at 25°C

Brain Heart CC Agar

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g
Bacto Agar	15 g
Chloramphenicol	50 mg
Cycloheximide	500 mg

Final pH 7.4 ± 0.2 at 25°C

Clostridium Difficile Antimicrobic Supplement CC

Formula per 5 ml

Cycloserine	125 mg
Cefoxitin	5 mg

Precautions

1. For Laboratory Use.
2. **Brain Heart CC Agar: HARMFUL.** HARMFUL 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, Cardiovascular, Muscles, Blood, Lymph Glands, 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. **Brain Heart CC Agar**: Avoid overheating or holding the medium in the melted state. Doing so tends to reduce the selective properties of the medium.
4. When testing human serum, treat all specimens as infectious agents.
5. 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 Clostridium Difficile Antimicrobic Supplement CC 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

Brain Heart Infusion
Brain Heart Infusion Agar
Clostridium Difficile Antimicrobic Supplement CC
Brain Heart CC Agar
Brain Heart Infusion w/PAB and Agar
Brain Heart Infusion w/o Dextrose

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Waterbath (optional)
Sterile defibrinated blood (optional)
Sterile Petri dishes
Sterile tubes
Anaerobic system for Clostridium Difficile Agar

Method of Preparation

Brain Heart Infusion Media

1. Suspend an appropriate amount of the selected medium in 1 liter distilled or deionized water:
   Brain Heart Infusion - 37 grams;
   Brain Heart Infusion Agar - 52 grams;
   Brain Heart CC Agar - 52 grams;
   Brain Heart Infusion w/PAB and Agar - 38 grams;
   Brain Heart Infusion w/o Dextrose - 35 grams.
2. If the medium contains agar (Brain Heart Infusion Agar, Brain Heart CC Agar and Brain Heart Infusion w/PAB and Agar), heat it to boiling to dissolve completely. Avoid overheating.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Clostridium Difficile Agar

1. Rehydrate and sterilize 500 ml of **Brain Heart Infusion Agar** per label directions. Cool to 45-50°C.
2. Aseptically rehydrate **Clostridium Difficile Antimicrobic Supplement CC** with 5 ml sterile distilled or deionized water. Invert the vial gently several times to dissolve the contents. Use immediately.
3. Aseptically add 5% sterile defibrinated sheep blood or 7% defibrinated horse blood and 5 ml of Clostridium Difficile Antimicrobic Supplement CC to the rehydrated medium.
4. Mix thoroughly, avoiding the formation of bubbles, and dispense into sterile Petri dishes.

Specimen Collection and Preparation

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

Test Procedure

See appropriate references for specific procedures using Brain Heart Infusion Media.

Clostridium Difficile Agar

1. Inoculate a representative portion of the specimen directly onto the surface of a freshly prepared or previously reduced Clostridium Difficile Agar plate and streak for isolation. The inoculum should include mucous, blood or membranous material, if present.
2. Incubate at 35°C under anaerobic conditions.
3. Examine for growth after 24-48 hours incubation.

   For a complete discussion on the isolation and identification of *Clostridium difficile* refer to appropriate procedures in the references.[15,18,20]

Results

Clostridium Difficile Agar

After 24 hours incubation, colonies of *C. difficile* appear non-hemolytic, 1-3 mm in diameter, off-white to gray, flat and circular with an undulated edge. Colonies become larger (3-5 mm) after 48 hours incubation.

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. Certain pathogenic fungi may be inhibited by the antibiotics in Brain Heart CC Agar.[19]
3. Clostridium Difficile Antimicrobic Supplement CC is intended for use in the preparation of Clostridium Difficile Agar. Although this medium is selective for *C. difficile*, additional testing using pure cultures is necessary for complete identification. Consult appropriate references for further information.[15,18,20]
4. Suspected colonies of *C. difficile* should be Gram stained and subcultured anaerobically and aerobically on blood agar for complete identification.
5. Demonstration of the *C. difficile* toxin in feces in the presence of clinically evident pseudomembranous colitis is required for definitive diagnosis.[20]

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Research **1**:205- 249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Roseburg, T., L. J. Epps, and A. R. Clark.** 1944. A study of the isolation, cultivation and pathogenicity of *Actinomyces israeli* recovered from the human mouth and from actinomycosis in man. J. Infect. Dis. **29**:390.
4. Howell, A. 1948. The efficiency of methods for the isolation of *Histoplasma capsulatum*. Public Health Reports **63**:173-178.
5. **Willey, S. H., and J. G. Bartlett.** 1979. Cultures for *Clostridium difficile* in stools containing a cytotoxin neutralized by *Clostridium sordellii* antitoxin. J. Clin. Microbiol. **6**:880-884.
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7. **Ajello, L., L. K. Georg, W. Kaplan, and L. Kaufman**. 1966. Laboratory manual for medical mycology (CDC), U.S. DHEW, Center for Disease Control, Atlanta, GA.

8. **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.
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10. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
11. **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.
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13. **National Committee for Clinical Laboratory Standards.** 1994. M11-A3, Vol. 13, No. 26, Methods for antimicrobial susceptibility testing of anaerobic bacteria. National Committee for Clinical Laboratory Standards, Villanova, PA.
14. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 147-153, CRC Press, Boca Raton, FL.
15. **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.
16. **Swenson, J. M., N. C. Clark, M. J. Ferraro, D. F. Sahm, G. Doern, M. A. Pfaller, L. B. Reller, M. P. Weinstein, R. J. Zabransky, and F. C. Tenover.** 1994. Development of a standardized screening method for detection of vancomycin-resistant enterococci. J. Clin. Microbiol. **32**:1700-1704.
17. **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**:116-119.
18. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
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20. **Onderdonk, A. B., and S. D. Allen.** 1995. *Clostridium*, p. 574 -586. *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.

Packaging

Brain Heart Infusion	100 g	0037-15
	500 g	0037-17
	2 kg	0037-07
	10 kg	0037-08
Brain Heart Infusion Agar	100 g	0418-15
	500 g	0418-17
	2 kg	0418-07
Brain Heart CC Agar	500 g	0483-17
Brain Heart Infusion w/PAB and Agar	500 g	0499-17
Brain Heart Infusion w/o Dextrose	10 kg	0502-08
Clostridium Difficile Antimicrobic Supplement CC	6 x 5 ml	3194-57*

*Store at 2-8°C

Bacto® Brain Heart Infusion, Porcine

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water. Light to medium amber, clear.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	fair
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

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

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

Intended Use

Bacto Brain Heart Infusion, Porcine is used for cultivating a wide variety of microorganisms.

Also Known As

Brain Heart Infusion, Porcine is abbreviated as BHI, Porcine.

Summary and Explanation

Rosenow[1] devised an excellent medium for culturing streptococci by supplementing Dextrose Broth with brain tissue. Hayden,[2] revising Rosenow's procedure by adding crushed marble to the medium, reported favorable growth of organisms from dental pathogens. Brain Heart Infusion (0037) is a modification of the media described by Rosenow[1] and Hayden.[2] Infusion from calf brains has replaced the brain tissue and Disodium Phosphate has replaced the Calcium Carbonate buffer.

Brain Heart Infusion, Porcine was developed as an alternative to Brain Heart Infusion formula, and replaces calf brains and beef heart with porcine brains and heart. Brain Heart Infusion, Porcine was developed for pharmaceutical and vaccine production and can replace the traditional BHI depending on organism and production application.

BHI, Porcine was formulated with no bovine components to minimize Bovine Spongiform Encephalopathy (BSE) risk.

The nutritionally rich formula of BHI is used to grow a variety of microorganisms The original Brain Heart Infusion media are specified in standard methods for multiple applications.[3,4,5,6]

Principles of the Procedure

Infusion from pork brains, infusion from pork heart and Pork Peptone No. 2 provides nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion, Porcine. Dextrose is the carbon energy source to facilitate organism growth. Sodium Chloride maintains the osmotic balance of the medium. Disodium Phosphate is the buffering agent.

Formula

Brain Heart Infusion, Porcine

Formula Per Liter	
Pork Brains, Infusion from	200 g
Pork Heart, Infusion from	250 g
Bacto Pork Peptone No. 2	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 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

Brain Heart Infusion, Porcine

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Waterbath (optional)

Method of Preparation

1. Dissolve 37 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Dispense as desired.

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 Brain Heart Infusion.

Results

Refer to appropriate references and procedures for results.

References

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

Packaging

Brain Heart Infusion, Porcine	500 g	0561-17

Bacto® Brewer Anaerobic Agar

Intended Use

Bacto Brewer Anaerobic Agar is used for cultivating anaerobic and microaerophilic bacteria.

Summary and Explanation

Brewer[1] described a special Petri dish cover that allowed surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on agar with a low oxidation-reduction potential. Brewer Anaerobic Agar was originally formulated and modified for the procedure described by Brewer.[1] This medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.[2,3,4]

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

Principles of the Procedure

Tryptone, Proteose Peptone No. 3 and Yeast Extract provide the nitrogen, vitamins and amino acids in Brewer Anaerobic Agar. Dextrose

is the carbon source, and Sodium Chloride maintains osmotic equilibrium. Sodium Thioglycollate and Sodium Formaldehyde Sulfoxylate are the reducing agents. Resazurin serves as an indicator of anaerobiosis with a pink color indicating the presence of oxygen. Bacto Agar is the solidifying agent.

Formula

Brewer Anaerobic Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Sodium Chloride	5 g
Bacto Agar	20 g
Sodium Thioglycollate	2 g
Sodium Formaldehyde Sulfoxylate	1 g
Resazurin, Certified	0.002 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.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in distilled or deionized water on boiling. Light amber, slightly opalescent while hot, turning red on aeration and cooling.
Prepared Medium:	Light pink ring at outer edge, light amber in center, slightly opalescent.
Reaction of 5.8% Solution at 25°C	pH 7.2 ± 0.2

Cultural Response

Prepare Brewer Anaerobic Agar per label directions. Inoculate the plates using the streak method. Incubate plates at 35 ± 2°C anaerobically for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis	25285*	100-1,000	good
Clostridium beijerinckii	17795	100-1,000	good
Clostridium perfringens	12924	100-1,000	good

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

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

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

Brewer Anaerobic Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 58 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense as desired.

Specimen Collection and Preparation

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[2] Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

Standard Petri Dishes:[2]

1. Inoculate a properly obtained specimen onto the medium, and streak to obtain isolated colonies.
2. Immediately incubate anaerobically at 35 ± 2°C.
3. Examine at 24 hours if incubating plates in an anaerobic chamber. Examine at 48 hours if incubating plates in an anaerobic jar or pouch, or if using Brewer anaerobic dish cover.
4. Extended incubation may be necessary to recover some anaerobes.

Brewer Anaerobic Agar Plates:

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

For a complete discussion on anaerobic and microaerophilic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[2,3,4] For the examination of anaerobic bacteria in food refer to standard methods.[7,8,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[2]
3. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[2]
4. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure the organism is an anaerobe.[2]

References

1. **Brewer, J. H.** 1942. A new Petri dish and technique for use in the cultivation of anaerobes and microaerophiles. Science **95**:587.
2. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Etiological agents recovered from clinical material, p. 474-503. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
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6. **Smith, L. D. S.** 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, IL.
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9. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Brewer Anaerobic Agar	500 g	0279-17
	10 kg	0279-08

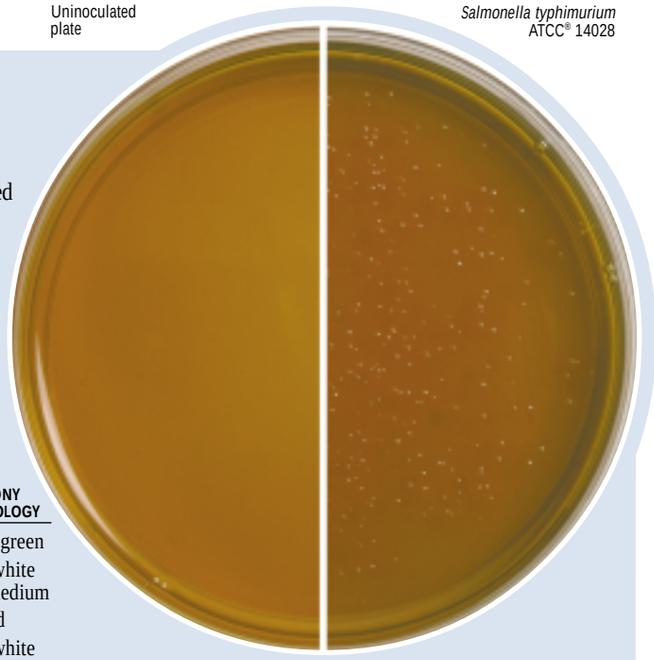
Bacto® Brilliant Green Agar

Intended Use

Bacto Brilliant Green Agar is used for isolating *Salmonella* other than *Salmonella typhi*.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella*



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	5.8% solution, soluble in distilled or deionized water on boiling. Solution is brownish-green, clear to very slightly opalescent.
Prepared Plates:	Orangish-brown, very slightly to slightly opalescent.
Reaction of 3.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY MORPHOLOGY
Escherichia coli	25922*	2,000-10,000	none to poor	yellow-green
Salmonella enteritidis	13076	100-1,000	good	pink-white w/red medium
Salmonella typhi	19430	100-1,000	none to poor	red
Salmonella typhimurium	14028*	30-300	good	pink-white w/red medium
Staphylococcus aureus	25923*	2,000-10,000	markedly 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.

in domesticated animals. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness.[1] The illness results from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many of these cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. Various poultry products are routinely monitored for *Salmonella* before their distribution for human consumption, but in many instances, contaminated food samples elude monitoring.

The use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella* was first described by Kristensen, Lester and Jurgens[2] who reported it useful for the differentiation of "paratyphoid B" from other intestinal gram-negative bacilli. Later, Kauffmann[3] modified their formula and used Brilliant Green Agar in addition to Tetrathionate Broth for the isolation of *Salmonella* from stool specimens. Gallon and Quan[4] increased their positive *Salmonella* findings by using Tetrathionate Broth and plating on Brilliant Green Agar. Broh-Kahn[5] showed that the Kauffmann modification of Brilliant Green Agar permitted the use of heavy inocula to obtain maximum recovery of *Salmonella* from fecal specimens. Miller and Tate[6] found that the addition of 20 mg per liter of sodium novobiocin to Brilliant Green Agar reduced or completely inhibited nuisance organisms commonly seen on agar media used for isolating salmonellae. Brilliant Green Agar with Novobiocin is also recommended for use when testing food for *Salmonella*.[7]

Brilliant Green Agar is recommended for use in testing clinical specimens.[8,9] The outstanding selectivity of this medium permits the use of moderately heavy inocula, which should be evenly distributed over the surface. Brilliant Green Agar is valuable when investigating outbreaks of *Salmonella* spp., other than *S. typhi* and *S. paratyphi*.[8,9] In addition, Brilliant Green Agar is used in the microbial limits test as recommended in the United States Pharmacopeia. The microbial limits test is performed to ensure that pharmaceutical articles are free of *Salmonella* spp.[10]

Principles of the Procedure

In Brilliant Green Agar, Proteose Peptone No. 3 and Yeast Extract provide nitrogen, vitamins and minerals. Lactose and Saccharose are the carbohydrates in the medium. Phenol Red is the pH indicator that turns the medium a yellow color with the formation of acid when lactose and/or sucrose is fermented. Sodium Chloride maintains the osmotic balance in the medium. Brilliant Green inhibits gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Lactose/sucrose fermenters are usually inhibited.[11] Bacto Agar is the solidifying agent.

Formula

Brilliant Green Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Bacto Saccharose	10 g
Sodium Chloride	5 g
Bacto Agar	20 g
Brilliant Green	0.0125 g
Bacto Phenol Red	0.08 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Brilliant Green Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 58 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Cool to 45-50°C in a waterbath.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7,8,9]
2. For specific information about specimen preparation and inoculation of clinical specimens, consult the appropriate references.[7,8,9]

Test Procedure

For isolation of *Salmonella* from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the Brilliant Green Agar plate and streak for isolation. This will permit the development of discrete colonies. Incubate plates at 35°C. Examine plates after 18-24 hours for colonies with characteristic morphologies associated with *Salmonella* spp.

Results

The typical *Salmonella* colonies appear as pink-white opaque colonies surrounded by a brilliant red medium. The few lactose and/or sucrose fermenting organisms that grow are readily differentiated due to the formation of a yellow-green colony surrounded by an intense

yellow-green zone. Brilliant Green Agar is not suitable for the isolation of *S. typhi* or *Shigella*; however, some strains of *S. typhi* may grow forming red colonies.

Limitations of the Procedure

1. Colonies of *Salmonella* spp. vary from red-pink-white depending on length of incubation and strain.[11]
2. Medium is normally orangish-brown in color; however, on incubation, it turns bright red but returns to normal color at room temperature.[11]
3. Studies by Taylor[12] showed that slow lactose fermenters, *Proteus*, *Citrobacter*, and *Pseudomonas* may grow on Brilliant Green Agar as red colonies.
4. In routine examination of clinical specimens or other materials for the gram-negative intestinal pathogens, other primary plating media such as MacConkey Agar, and fluid enrichments such as Tetrathionate Broth and Selenite Broth, should be used with Brilliant Green Agar.
5. *S. typhi* does not grow adequately on this medium. *Shigella* spp. do not grow.[11]

References

1. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall, (ed.). Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Kristensen, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **Galton, M. M., and M. S. Quan.** 1944. *Salmonella* isolated in Florida during 1943 with the combined enrichment method of Kauffmann. Am. J. Public Health **34**:1071.
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6. **Tate, C. R., and R. G. Miller.** 1990. Modification of brilliant green agar by adding sodium novobiocin to increase selectivity for *Salmonella*. The Maryland Poultryman **4**:7-11.
7. **Federal Register.** 1993. Chicken Disease Caused by *Salmonella enteritidis*; proposed rule. Fed. Regis. **58**:41048-41061.
8. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.1-1.20.47. *In* H. D.Isenberg, (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
9. **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.
10. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
11. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
12. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars: New media for isolation of enteric pathogens. Am. J. Clin. Pathol. **44**:471.

Packaging

Brilliant Green Agar	100 g	0285-15
	500 g	0285-17

Bacto® Brilliant Green Agar Modified

Intended Use

Bacto Brilliant Green Agar Modified is used for isolating *Salmonella* from water, sewage and foodstuffs.

Summary and Explanation

Kampelmacher[1] proposed the formula for a selective medium to isolate *Salmonella* from pig feces and minced meat. Brilliant Green Agar Modified is more selective than Desoxycholate Citrate Agar and other brilliant green media, and inhibits the growth of *Pseudomonas aeruginosa* and *Proteus* sp. which may resemble *Salmonella*. *Salmonella cholerasuis* grows well on Brilliant Green Agar Modified, but poorly on Desoxycholate Citrate Agar.[2]

Brilliant Green Agar Modified is recommended for the isolation of *Salmonella*, other than *Salmonella typhi*, from water and associated materials[3] and meat and meat products.[4] It is recommended by the British Poultry Meat Society[5] for the examination of poultry and poultry products. The recommended procedures include using complementary selective culture media and techniques to increase the likelihood of isolating multiple serotypes of *Salmonella* from samples.[6]

Principles of the Procedure

Brilliant Green Agar Modified contains Beef Extract and Bacto Peptone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Lactose and Sucrose are carbohydrate sources. In the presence of Phenol Red, a pH indicator, nonlactose and/or nonsucrose-fermenting *Salmonella* will produce red colonies. Brilliant Green inhibits gram positive organisms and many gram negative bacteria, except *Salmonella*. Bacto Agar is a solidifying agent.

Formula

Brilliant Green Agar Modified

Formula Per Liter

Bacto Beef Extract	5 g
Bacto Peptone	10 g
Bacto Yeast Extract	3 g
Disodium Hydrogen Phosphate	1 g
Sodium Dihydrogen Phosphate	0.6 g
Lactose	10 g
Sucrose	10 g
Phenol Red	0.09 g
Brilliant Green	0.0047 g
Bacto Agar	12.0 g

Final pH 6.9 ± 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 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

Brilliant Green Agar Modified

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (42°C)
Sterile Blender Jar
Buffered Peptone Water
Muller Kauffmann Tetrathionate Broth
Selenite Brilliant Green Medium

Method of Preparation

1. Suspend 52 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. DO NOT AUTOCLAVE.

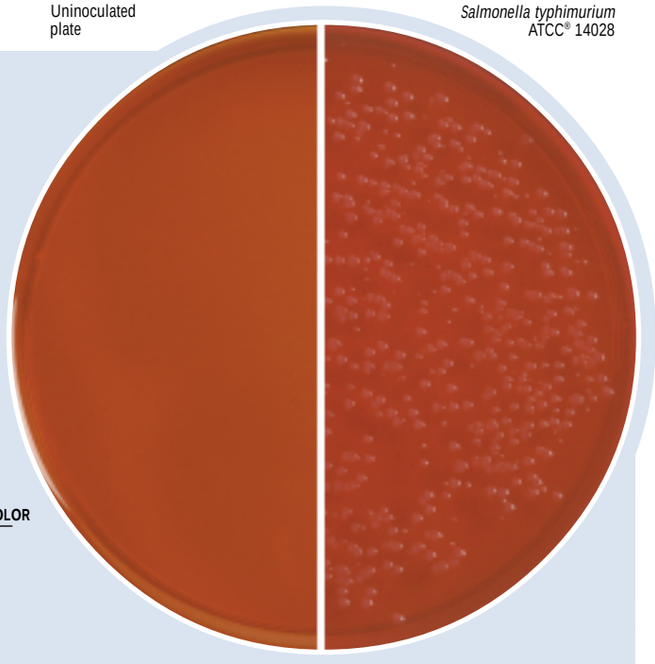
Specimen Collection and Preparation

Meat and Meat Products

1. Weigh 25 g of the sample into a sterile blender jar and add 225 ml of Buffered Peptone Water. Macerate for a sufficient time to give 15,000-20,000 revolutions.
2. Aseptically transfer the contents of the blender jar to a 500 ml flask. Incubate at 37 ± 0.1°C for 16-20 hours.
3. Transfer 10 ml samples to 100 ml Muller Kauffmann Tetrathionate Broth and to 100 ml of Selenite Brilliant Green Medium.
4. Incubate the Muller Kauffmann Tetrathionate Broth at 42-43°C and the Selenite Brilliant Green Enrichment at 37°C.

Sewage Polluted Natural Water

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



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling. Solution is orange-brown, clear to slightly opalescent.
Prepared Medium:	Orange-brown, clear to slightly opalescent.
Reaction of 5.2% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	1,000-2,000	completely to partially inhibited	green
Proteus mirabilis NCTC	11938	1,000-2,000	completely to partially inhibited	red
Salmonella typhimurium	14028*	100-1,000	good	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.

1. Inoculate 25 ml aliquots of the sample into 25 ml of double strength Buffered Peptone Water (1810) and incubate at 37°C for 18 hours.
2. Transfer 1 ml samples into 10 ml of Muller Kauffmann Tetrathionate Broth.
3. Incubate at 43°C for 48 hours.

Test Procedure

1. Subculture the broths at 18-24 hours and at 48 hours onto Brilliant Green Agar (Modified).
2. Examine for typical colonies of *Salmonella* after overnight incubation at 37°C.

Results

Salmonella will produce red colonies.

Limitations of the Procedure

1. Due to the nutritional requirements and inhibitory characteristics of the organisms themselves, organisms other than *Salmonella* spp., such as *Morganella morgani* and some *Enterobacteriaceae* may grow on the medium.
2. Confirmatory tests, such as fermentation reactions and seroagglutination, should be carried out on all presumptive *Salmonella* spp.

References

1. **Guinee, P. A., and E. H. Kampelmacher.** 1962. Antonie van Leeuwenhoek. **28**:417-427.
2. **Heard, T. W., N. E. Jennet, and A. H. Linton.** 1969. British Veterinary Journal **125**:635-644.
3. **H. M. S. O.** 1982. Methods for the isolation and identification of salmonellae (other than *Salmonella typhi*) from water and associated materials.
4. **International Organisation for Standardisation.** 1974. Draft International Standard ISO/DIS 3565. Geneva.
5. **British Poultry Meat Society.** 1982. A manual of recommended methods for the microbiological examination of poultry and poultry products.
6. **Harvey, R. W. S., and T. H. Price.** 1976. J. Hygiene Camb. **77**:333-339.

Packaging

Brilliant Green Agar Modified	500 g	1880-17

Bacto® Brilliant Green Bile Agar

User Quality Control

Identity Specifications

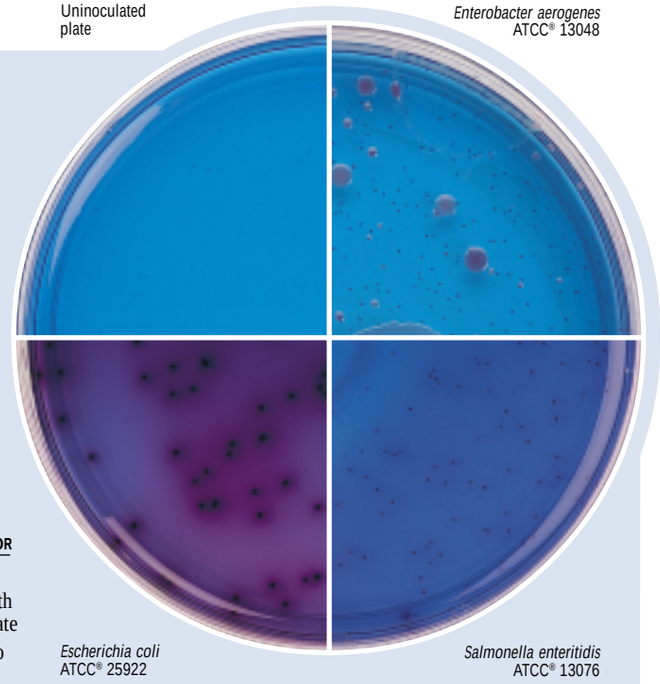
Dehydrated Appearance: Light purple, free-flowing, homogeneous.

Solution:	2.06% solution, soluble in distilled or deionized water on boiling. Solution is bluish purple, very slightly to slightly opalescent.
Prepared Medium:	Bluish purple, slightly opalescent.
Reaction of 2.06% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Enterobacter aerogenes	13048*	100-1,000	good	pink
Escherichia coli	25922*	100-1,000	good	deep red with bile precipitate
Salmonella enteritidis	14028	100-1,000	good	colorless to light pink
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition	–



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

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

Intended Use

Bacto Brilliant Green Bile Agar is used for isolating, differentiating and enumerating coliform bacteria.

Also Known As

Brilliant Green Bile Agar (BGBA) is also known as Brilliant Green Agar[2] (BGA).

Summary and Explanation

Noble and Tonney[1] described Brilliant Green Bile Agar for determining the relative density of coliform bacteria in water and sewage. The medium is particularly useful in selectively isolating *Salmonella* spp. from other coliform bacteria. The American Public Health Association (APHA) specifies a qualitative procedure to isolate and identify *Salmonella* spp. from water and wastewater using concentration, enrichment and selective growth.[2]

Principles of the Procedure

Brilliant Green Bile Agar contains Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Lactose is a fermentable carbohydrate. Oxgall (bile) and brilliant green inhibit gram-positive bacteria and most gram-negative bacteria except coliforms. Basic Fuchsin is a pH indicator. Monopotassium phosphate is a buffering agent. Agar Noble is a solidifying agent.

Differentiation of the coliforms is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of basic fuchsin, form deep red colonies with a pink halo. Bacteria that do not ferment lactose form colorless to faint pink colonies. Coliform bacteria typically ferment lactose, producing deep red colonies, while *Salmonella* spp., which do not ferment lactose, produce colorless to faint pink colonies.

Formula

Brilliant Green Bile Agar

Formula Per Liter

Bacto Peptone	8.25 g
Bacto Lactose	1.9 g
Bacto Oxgall	0.00295 g
Sodium Sulfite	0.205 g
Ferric Chloride	0.0295 g
Monopotassium Phosphate	0.0153 g
Agar Noble	10.15 g
Erioglaucine	0.0649 g
Bacto Basic Fuchsin	0.0776 g
Brilliant Green	0.0000295 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

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

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

Storage

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

This medium is light sensitive. Protect from exposure to direct sunlight.

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

Brilliant Green Bile Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°)

Method of Preparation

1. Suspend 20.6 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 room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. The medium is sensitive to light, particularly direct sunlight, which produces a decrease in the productivity of the medium and a change in color from deep blue to purple or red. The medium should be prepared just prior to use and, when necessary to store the medium, it should be kept in the dark.

References

1. **Nobel and Tonney**. 1935. J. Am. Water Works Assoc. **27**:108.
2. **Eaton, 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

Brilliant Green Bile Agar	500 g	0014-17

Bacto® Brilliant Green Bile 2%

Intended Use

Bacto Brilliant Green Bile 2% is used for confirming the presence of coliform organisms in water and foods.

Also Known As

Brilliant Green Bile Broth
Brilliant Green Lactose Bile Broth, 2%
Brilliant Green Lactose Bile Broth
Brilliant Green Bile Lactose Broth

Summary and Explanation

The coliform group of bacteria includes aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Members of the *Enterobacteriaceae* comprise the majority of this group but organisms such as *Aeromonas* species may also be included.

Procedures to detect and confirm coliforms are used in testing water, foods, dairy products and other materials.[1,2,3,4,5] The procedures begin with a presumptive test that, when positive, is confirmed by using Brilliant Green Bile 2%.

Principles of the Procedure

Bacto Peptone is a source of carbon and nitrogen for general growth requirements. Oxgall (bile) and Brilliant Green inhibit gram-positive bacteria and many gram-negative bacteria other than coliforms. Lactose is a carbohydrate source. Bacteria that ferment lactose and produce gas are detected.

Formula

Brilliant Green Bile 2%

Formula Per Liter

Bacto Peptone	10 g
Bacto Oxgall	20 g
Bacto Lactose	10 g
Brilliant Green	0.0133 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable gloves and eye/face protection. Use only in well ventilated areas. Keep container tightly closed. TARGET ORGAN(S): Lungs.

   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:	Greenish-beige, free-flowing, homogeneous.
Solution:	4.0% solution soluble in distilled or deionized water on warming, if necessary; emerald green, clear without significant precipitate.
Prepared Medium:	Emerald green, clear.
Reaction of 4.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Brilliant Green Bile 2% per label directions. Inoculate medium and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	GAS PRODUCTION
Enterobacter aerogenes	13048*	100-1,000	good	+
Escherichia coli	25922*	100-1,000	good	+
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition	–
Enterococcus faecalis	19433	1,000-2,000	marked to complete inhibition	–

Uninoculated tube — *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.

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

Brilliant Green Bile 2%

Materials Required but not Provided

Flask with closure
Test tubes with caps
Fermentation tubes
Distilled or deionized water
Autoclave
Incubator

Method of Preparation

1. Suspend 40 grams in 1 liter distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Dispense required amount in tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Process specimens according to established procedures for the type of material being tested.[1,2,3,4,5]

Test Procedure

Consult standard references for specific instructions for the type of material being tested.[1,2,3,4,5]

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

Results

Positive: Bubbles (gas) in fermentation tube.

Negative: No bubbles (gas) in fermentation tube.

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. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1993. Coliform and other indicator bacteria, p. 247-269. *In* R. T. Marshall (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **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 **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler.** 1995. *Escherichia coli* and the coliform bacteria, p. 4.01-4.29. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Andrews, W. H.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.

Packaging

Brilliant Green Bile 2%	100 g	0007-15-6
	500 g	0007-17-4
	2 kg	0007-07-6
	10 kg	0007-08-5

Bacto® mBrilliant Green Broth

Intended Use

Bacto mBrilliant Green Broth is used for recovering and differentiating *Salmonella* from primary water samples by membrane filtration.

Summary and Explanation

mBrilliant Green Broth is primarily used as a selective-differential medium for *Salmonella* species. *Salmonella* species cause many types of infections from mild, self-limiting gastroenteritis to life-threatening typhoid fever.[4] The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than two days and diarrhea lasting less than 7 days.[4]

mBrilliant Green Broth is a modification of Kauffmann's[1] Brilliant Green Agar in which the agar has been omitted and all other ingredients are at double strength.

Kabler and Clark[2] used mBrilliant Green Broth in a membrane filtration procedure originally developed by Geldreich and Jeter.[3] In this technique, an appropriate volume of water is filtered through the membrane filter. The filter is placed on an absorbent pad saturated with m Tetrathionate Broth Base. After incubation, the membrane is transferred to another absorbent pad saturated with mBrilliant Green Broth and incubated. Following incubation, the membrane is transferred to a fresh pad saturated with urease test reagent.

Principles of the Procedure

Proteose Peptone No. 3 provides the nitrogen, minerals and amino acids in mBrilliant Green Broth. Yeast Extract is the vitamin source. Lactose and Saccharose are the carbohydrates for bacterial growth. Sodium Chloride maintains the osmotic balance of the medium and Phenol Red is the dye used as an indicator of carbohydrate fermentation. Brilliant Green is the selective agent.

Formula

mBrilliant Green Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Bacto Yeast Extract	6 g
Bacto Lactose	20 g
Bacto Saccharose	20 g
Sodium Chloride	10 g
Bacto Phenol Red	0.16 g
Brilliant Green	0.025 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.

Use the rehydrated medium within 24 hours.

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

mBrilliant Green Broth

Materials Required But Not Provided

Glassware
Sterile absorbent pad
Membrane filtration equipment
Incubator (35°C)
Sterile Petri dishes, 50 x 9 mm
Distilled or deionized water

Method of Preparation

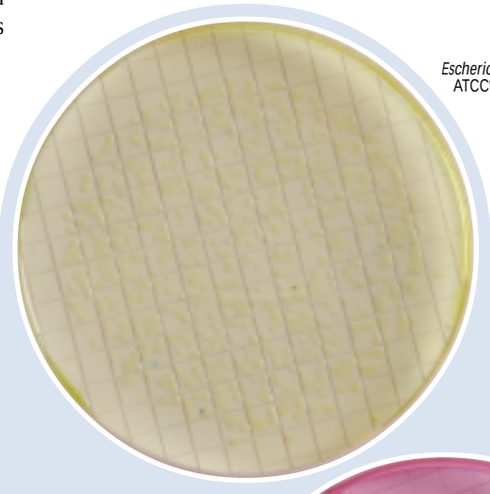
1. Suspend 7.6 grams in 100 ml of distilled or deionized water.
2. Heat to boiling to dissolve completely. Do not autoclave.
3. Cool to room temperature.
4. Dispense 2 ml amounts onto sterile absorbent pads.
5. Use the rehydrated medium within 24 hours.

Specimen Collection and Preparation

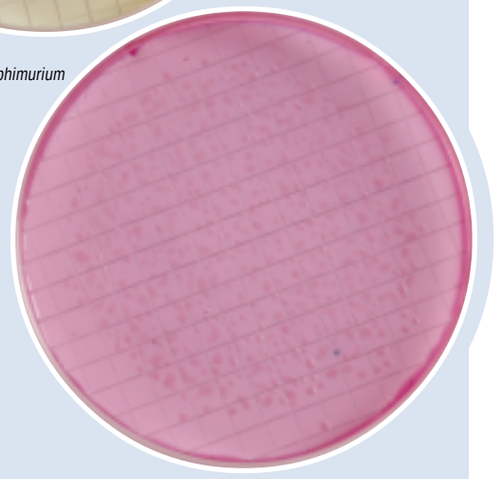
Obtain and process water samples according to the techniques and procedures established by laboratory policy.

Test Procedure

1. Inoculate a water sample using the membrane filtration procedure.
2. Place the filter on a pad saturated with mBrilliant Green Broth.
3. Incubate at 35 ± 2°C in a humid atmosphere for 18-24 hours.
4. After incubation, examine for growth and the color of the colonies.



Escherichia coli
ATCC® 25922



Salmonella typhimurium
ATCC® 14028

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	7.6% solution, soluble in distilled or deionized water; greenish-red, slightly opalescent.
Prepared Medium	Greenish-red, slightly opalescent.
Reaction of 7.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare mBrilliant Green Broth per label directions. Inoculate using the membrane filter technique and incubate at 35 ± 2°C in a humid atmosphere for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR OF COLONY
Escherichia coli	25922*	20-80	good	yellow
Salmonella enteritidis	13076	20-80	good	pink to red
Salmonella typhimurium	14028*	20-80	good	pink to 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.

Results

Salmonella species form pink to red colonies.

Limitations of the Procedure

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

References

1. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren fur Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
2. **Kabler and Clark.** 1952. Am. J. Public Health **42**:390.
3. **Geldreich and Jeter.** 1952. Bacteriol. Proc. SAB., Boston, MA.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

mBrilliant Green Broth	500 g	0494-17

Bacto® Brucella Agar
Bacto Brucella Broth

Intended Use

Bacto Brucella Agar is used for isolating and cultivating *Brucella*.

Bacto Brucella Broth is used for cultivating *Brucella* and other fastidious microorganisms.

User Quality Control

Identity Specifications

Brucella Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.3% solution, soluble in distilled or deionized water with frequent agitation on boiling. Light amber, very slightly to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 4.3% Solution at 25°C:	pH 7.0 ± 0.2

Brucella Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.8% solution, soluble in distilled or deionized water. Light amber, clear to very slightly opalescent, no precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent, no precipitate.
Reaction of 2.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Brucella Agar or Brucella Broth per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 24-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella abortus	4315	100-1,000	good
Brucella melitensis	4309	100-1,000	good
Brucella suis	4314	100-1,000	good

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

Summary and Explanation

Brucella Agar and Brucella Broth are prepared according to the APHA formula for Albimi Broth, which is used for the isolation of *Brucella* species.[1] Brucellosis is a zoonotic disease with a domestic-animal reservoir.[2] Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[2]

Brucella Agar is used as a general medium for the cultivation of fastidious microorganisms, e.g., *Streptococcus pneumoniae*, *Streptococcus viridans* and *Neisseria meningitidis*.[3] With the addition of blood, Brucella Agar can be used to determine hemolytic reactions of pathogenic bacteria.[3] Brucella Agar can be used as a base for the isolation of *Campylobacter* species.[3]

Brucella Broth is recommended for the isolation of *Brucella* species from blood cultures.[4,5] Brucella Broth is specified in the Compendium of Methods for the Microbiological Examination of Food.[6]

Principles of the Procedure

Peptamin provides nitrogen and amino acids. Tryptone provides nitrogen. Yeast Extract adds essential vitamins. Dextrose is a carbon source; Sodium Bisulfite enhances growth. Sodium Chloride maintains the osmotic balance. Bacto Agar is the solidifying agent in Brucella Agar.

Supplemental blood (5-10%) provides additional growth factors for fastidious microorganisms and is used to determine hemolytic reactions.

Formula

Brucella Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Peptamin	10 g
Bacto Dextrose	1 g
Bacto Yeast Extract	2 g
Sodium Chloride	5 g
Sodium Bisulfite	0.1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Brucella Broth

Formula Per Liter

Bacto Tryptone	10 g
Bacto Peptamin	10 g
Bacto Dextrose	1 g
Bacto Yeast Extract	2 g
Sodium Chloride	5 g
Sodium Bisulfite	0.1 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. *Brucella* species are classified as Biosafety Level 3 pathogens. All manipulations with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).[2]
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

Brucella Agar
Brucella Broth

Materials Required But Not Provided

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

Method of Preparation

1. **Brucella Agar**: Suspend 43 grams in 1 liter distilled or deionized water and boil to dissolve completely.

   Brucella Broth: Dissolve 28 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to 45-55°C.
3. OPTIONAL: To prepare Brucella Blood Agar, aseptically add 5-10% sterile defibrinated blood at 45-50°C. Mix well.

Specimen Collection and Preparation

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

Test Procedure

For a complete discussion of the isolation and identification of *Brucella*, refer to appropriate procedures outlined in the references.[2,4,5]

Results

Refer to apropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Hemolytic reactions of many microorganisms are different on horse blood from those on sheep blood agar; e.g., some Group D streptococci exhibit beta hemolysis on horse blood but not on sheep blood and are mistaken for Group A.[3]

References

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

Packaging

Brucella Agar	500 g	0964-17
	2 kg	0964-07
	10 kg	0964-08
Brucella Broth	500 g	0495-17
	10 kg	0495-08

Bacto® Bryant and Burkey Medium

Intended Use

Bacto Bryant and Burkey Medium is used for detecting and enumerating spores of lactate-fermenting *Clostridium* in milk and dairy products.

Summary and Explanation

Bryant and Burkey Medium is based on the lactate fermentation media described by Rosenberger[1] and Bryant and Burkey[2], as modified by Bergère et al,[3] who reported that their medium could be used for detecting and enumerating *C. tyrobutyricum* spores in milk and dairy products.[3-5]

Principles of the Procedure

Tryptone, Yeast Extract, Beef Extract Desiccated and L-Cysteine Hydrochloride provide nutrients and cofactors required for good growth of clostridia. Selectivity of this medium is achieved through the addition of Sodium Acetate, which is also the principal promoter of

germination by *C. tyrobutyricum* spores.[6] Sodium Lactate is fermented under anaerobic conditions by *C. tyrobutyricum* and other lactate-fermenting clostridia, producing hydrogen and carbon dioxide. Gas production is demonstrated by an upward movement of a paraffin plug which is overlaid on the medium. Resazurin is included in the medium to show anaerobiosis, turning from pink (aerobic) to colorless under anaerobic conditions.

During processing, the sample is heated at 75°C for 15 minutes to kill vegetative cells and activate germination of spores.

Formula

Bryant and Burkey Medium

Formula Per Liter

Bacto Tryptone	15 g
Bacto Yeast Extract	5 g
Bacto Beef Extract, Desiccated	7.5 g
Sodium Acetate	5 g
L-Cysteine Hydrochloride	0.5 g
Sodium Lactate	5 g
Resazurin	0.0025 g

Final pH 5.9 ± 0.2 at 25°C

Precautions

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear when hot, becoming red upon cooling.
Reaction of 3.8% Solution at 25°C:	pH 5.9 ± 0.2

Cultural Response

Prepare Bryant and Burkey Medium per label directions. Inoculate using Most Probable Number (MPN) method and incubate at 35 ± 2°C for 6 days.

ORGANISM	ATCC® OR STRAIN	GAS INOCULUM	GROWTH	PRODUCTION
Clostridium tyrobutyricum	CNRZ 500	MPN method	good	>1 cm of gas
Clostridium tyrobutyricum	CNRZ 510	MPN method	good	>1 cm of gas
Clostridium tyrobutyricum	CNRZ 608	MPN method	good	>1 cm of gas
Clostridium tyrobutyricum	25755	MPN method	good	>1 cm of gas

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

Storage

1. Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.
2. 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

Bryant and Burkey Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35 ± 2°C)

Method of Preparation

NOTE: This product contains sodium lactate; it is not necessary to add sodium lactate during preparation.

1. Dissolve 38 grams in 1 liter of distilled or deionized water.
2. Dispense 10 ml amounts into tubes.
3. Autoclave at 121°C for 15 minutes.

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

Three-tube Most Probable Number (MPN) Method

1. Before use, heat tubes to boiling for 10 minutes to regenerate anaerobic conditions. Note: This step is required only with tubes stored under aerobic conditions. Tubes stored under anaerobic conditions or freshly sterilized tubes do not need additional heating.
2. Prepare 10-fold dilutions of the sample and inoculate triplicate tubes of Bryant and Burkey Medium with 1 ml of each sample dilution.
3. Pour approximately 2 ml of melted paraffin (60-65°C), previously autoclaved at 121°C for 20 minutes, into each tube.
4. Heat the tubes at 75°C for 15 minutes to kill vegetative cells and activate spores; allow to cool to room temperature.
5. Incubate tubes at 35°C for 6 days.
6. Examine tubes for growth and gas production after 48 hours of incubation and daily for up to 6 days.

Results

Tubes showing both growth and production of gas (indicated by upward movement of the paraffin more than 1 cm) are considered positive for the presence of lactate-fermenting clostridial spores. Determine the spore count using the Most Probable Number (MPN) method.

References

1. **Rosenberger, K. F.** 1951. The development of methods for the study of obligate anaerobes in silage. Proc. Soc. Appl. Bacteriol. **14**:161-164.
2. **Bryant, M. P., and L. A. Burkey.** 1956. The characteristics of lactate-fermenting sporeforming anaerobes from silage. J. Bacteriol. **71**:43-46.
3. **Bergère, J. L., P. Gouet, J. Hermier, and G. Mocquot.** 1968. Les *Clostridium* du groupe butyrique dans les produits laitiers. Ann. Inst. Pasteur Lille. **19**:41-54.
4. **Cerf, O., and J. L. Bergère.** 1968. La numération des spores de *Clostridium* et son application au lait et aux produits laitiers. Numération des différents groupes de *Clostridium*. Le Lait **48**:501-519.
5. **Bergère, J. L.** 1979. Developpement de l'ensilage. Ses conséquences sur la qualité du lait et des produits laiters. Revue laitère française.
6. **Touraille, C., and J. L. Bergère.** 1974. La germination de la spore de *Clostridium tyrobutyricum*. Biochimie. **56**:404-422.

Packaging

Bryant and Burkey Medium	500 g	0645-17
	2 kg	0645-07

Bacto® Buffered Peptone Water
Bacto Modified Buffered Peptone Water

User Quality Control

Identity Specifications

Buffered Peptone Water

Dehydrated Appearance:	Cream-white to light tan, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 2.0% Solution at 25°C:	pH 7.2 ± 0.2

Modified Buffered Peptone Water

Dehydrated Appearance:	Light beige, free-flowing, homogenous.
Solution:	2.5% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 2.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Buffered Peptone Water or Modified Buffered Peptone Water per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Salmonella enteritidis	13076	10-100	good
Salmonella typhi	19430	10-100	good
Salmonella typhimurium	14028*	10-100	good

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

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

Intended Use

Bacto Buffered Peptone Water is used for preenriching damaged *Salmonella* species from food specimens to increase recovery.

Bacto Modified Buffered Peptone Water is used for preenriching *Salmonella* species from food specimens to increase recovery.

Summary and Explanation

Edel and Kampelmacher[1] noted that food preservation techniques involving heat, desiccation, preservatives, high osmotic pressure or pH changes cause sublethal injury to salmonellae. Preenrichment in a nonselective medium allows for repair of cell damage and facilitates the recovery of salmonellae. Lactose Broth is frequently used for this purpose but it may be detrimental to recovering salmonellae.[2] Buffered Peptone Water maintains a high pH over the preenrichment period and results in repair of injured cells that may be sensitive to low pH.[3] This is particularly important for vegetable specimens which have a low buffering capacity. These media can be used for testing dry poultry feed.[4] Buffered Peptone Water is a standard methods medium.[5]

Modified Buffered Peptone Water provides additional buffering capacity when organisms have been enriched in a pre-enrichment medium containing a high carbohydrate concentration.

Principles of the Procedure

Buffered Peptone Water and Modified Buffered Peptone Water contain Peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium Chloride maintains the osmotic balance. Phosphates buffer the medium.

Formula

Buffered Peptone Water

Formula Per Liter

Peptone	10 g
Sodium Chloride	5 g
Sodium Phosphate, Dibasic	3.5 g
Potassium Phosphate, Monobasic	1.5 g

Final pH 7.2 ± 0.2 at 25°C

Modified Buffered Peptone Water

Formula Per Liter

Peptone	10 g
Sodium Chloride	5 g
Sodium Phosphate, Dibasic	7 g
Potassium Phosphate, Monobasic	3 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Buffered Peptone Water**

   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.

   Modified Buffered Peptone Water

   IRRITANT. 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

Buffered Peptone Water
Modified Buffered Peptone Water

Materials Required but not Provided

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

Method of Preparation

1. Dissolve the medium in 1 liter distilled or deionized water:
   Buffered Peptone Water - 20 grams;
   Modified Buffered Peptone Water - 25 grams.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

Test specimens according to recommended guidelines.

Results

Growth is indicated by turbidity.

Limitations of the Procedure

1. The types and numbers of competing flora in the test sample can affect recovery and may overgrow salmonellae.

References

1. **Edel, W., and E. H. Kampelmacher.** 1973. Bull. World Hlth. Org. **48**:167-174.
2. **Angelotti, R**. 1963. Microbiological quality of foods. Academic Press, New York.
3. **Sadovski, A. Y**. 1977. J. Food Technol. **12**:85-91.
4. **Juven, B. J., N. A. Cox, J. S. Bailey, J. E. Thomson, O. W. Charles, and J. V. Schutze.** 1984. Recovery of *Salmonella* from artificially contaminated poultry feeds in non-selective and selective broth media. Jour. of Food Prot. **47**:299-302.
5. **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.

Packaging

Buffered Peptone Water	500 g	1810-17
	2 kg	1810-07
	10 kg	1810-08
Modified Buffered Peptone Water	500 g	1833-17

Bacto® Bushnell-Haas Broth

Intended Use

Bacto Bushnell-Haas Broth is used for studying microbial utilization of hydrocarbons.

Also Known As

Bushnell-Haas Broth is also referred to as Bushnell-Haas marine salts broth.

Summary and Explanation

Bushnell-Haas Broth, prepared according to the formula described by Bushnell and Haas[1], is used to evaluate the ability of microorganisms to decompose hydrocarbons. It is formulated without a carbon source which allows for the addition of alternate hydrocarbons such as kerosene, light and heavy mineral oils, paraffin wax, and gasoline.

Bushnell-Haas was recommended for the microbiological examination of fuels by the Society for Industrial Microbiology (SIM) Committee on Microbiological Deterioration of Fuels.[2] The medium was used to

enumerate total heterotrophs and hydrocarbon degradation by microorganisms during bioremediation of Prince William Sound following the Exxon *Valdez* oil spill.[3,4]

Principles of the Procedure

Magnesium Sulfate, Calcium Chloride, and Ferric Chloride provide trace elements necessary for bacterial growth. Potassium Nitrate is a nitrogen source, while Monopotassium Phosphate and Ammonium Phosphate Dibasic provide buffering capability.

Formula

Bushnell-Haas Broth

Formula Per Liter	
Magnesium Sulfate	0.2 g
Calcium Chloride	0.02 g
Monopotassium Phosphate	1 g
Ammonium Phosphate Dibasic	1 g
Potassium Nitrate	1 g
Ferric Chloride	0.05 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, Liver, 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.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige with pink tint, free-flowing, homogeneous.
Solution:	0.327% solution not soluble in distilled or deionized water, white precipitate remains. Solution, after autoclaving, is colorless to very light amber, clear supernatant over yellow-orange precipitate.
Prepared Medium:	Colorless to very light amber, clear supernatant over yellow-orange precipitate.
Reaction of 0.327% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Bushnell-Haas Broth per label directions. Inoculate in duplicate with the test organisms. Add sterile mineral oil (the hydrocarbon source) to one set. Incubate at 25-30°C for up to 1 week.

ORGANISM	ATCC®	INOCULUM	PLAIN	RECOVERY w/Hydrocarbon
Pseudomonas aeruginosa	9027	100-1,000	none to poor	good
Pseudomonas aeruginosa	10145	100-1,000	none to poor	good
Pseudomonas aeruginosa	14207	100-1,000	none to poor	good
Pseudomonas aeruginosa	27853*	100-1,000	none to poor	good

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

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

Storage

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

Bushnell-Haas Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (25-30°C)

Method of Preparation

1. Dissolve 3.27 grams in 1 liter distilled or deionized water.
2. Dispense as desired and autoclave at 121°C for 15 minutes.
3. Cool to 45-50ºC.

NOTE: A precipitate that is white prior to sterilization and turns yellow to orange after sterilization is normal.

Specimen Collection and Preparation

1. Collect samples in sterile containers or with sterile swabs and transport immediately to the laboratory.

Test Procedure

1. Inoculate the collected sample directly into the broth.
2. Overlay the broth with a sterile hydrocarbon source.
3. Incubate aerobically at 25-30°C.
4. Examine tubes daily for growth for up to one week.

Results

Organisms capable of degrading hydrocarbons should show growth in the Bushnell-Haas Broth supplemented with a hydrocarbon source.

Limitations of the Procedure

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

References

1. **Bushnell, L. D., and H. F. Haas.** 1941. The utilization of certain hydrocarbons by microorganisms. J. Bacteriol. **41**:653-673.

2. **Allred, R. C., R. J. DeGray, R. W. Edwards, H. G. Hedrick, D. E. Klemme, M. Rogers, M. Wulf, and H. Hodge.** 1963. Proposed procedures for microbiological examination of fuels. SIM Special Publications, Number 1. Merck, Sharp & Dohme Research Laboratories, Rahway, NJ.
3. **Bragg, J. R., J. C. Roffall, and S. McMillen.** 1990. Column flow studies of bioremediation in Prince William Sound. Exxon Production Research Co., Houston, TX.
4. **Brown, E. J., and J. F. Braddock.** 1990. Sheen Screen, a miniaturized most-probable-number method for enumeration of oil-degrading microorganisms. Appl. Environ. Microbiol. **56**:3895-3896.

Packaging

Bushnell-Haas Broth	500 g	0578-17
	10 kg	0578-08

Bacto® CLED Agar

Intended Use

Bacto CLED Agar is used for cultivating, differentiating and enumerating bacteria in urine.

Also Known As

CLED Agar is an abbreviation for Cystine Lactose-Electrolyte-Deficient Agar.

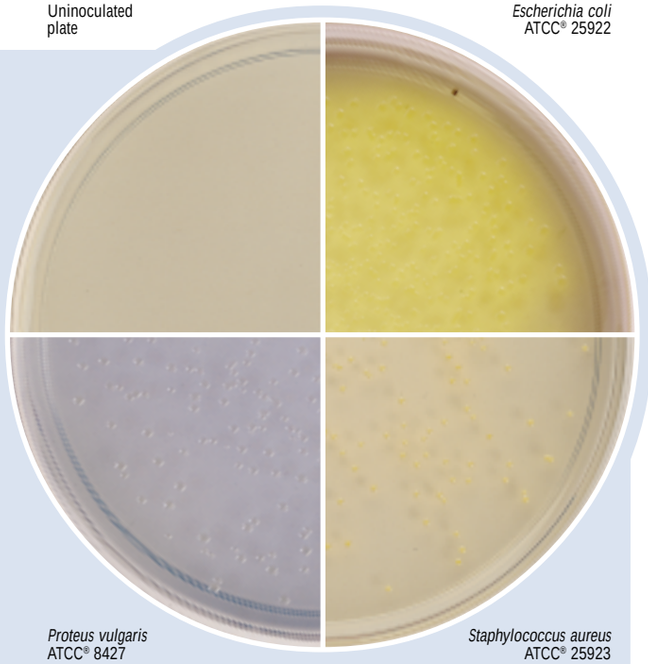
Summary and Explanation

Sandys[1] developed an electrolyte-deficient medium that prevented *Proteus* from swarming. Mackey and Sandys[2] modified the formula by substituting lactose and sucrose for mannitol, and increasing the amount of indicator and agar. While investigating this medium for a dip slide technique for urine cultures, the researchers further modified the formula. The revised formula omitted sucrose and added cysteine and was called Cystine Lactose-Electrolyte-Deficient medium.[3]

CLED Agar is recommended in the spread plate technique or as a dip slide for the detection of bacteria in urine. This medium supports the growth of urinary pathogens and provides distinct colony morphology. CLED medium lacks an electrolyte (salt) which is necessary for growth or other characteristics of certain bacteria.[4] Many European laboratories use Cystine Lactose-Electrolyte-Deficient (CLED) Agar.[5]

Principles of the Procedure

Beef Extract, Bacto Peptone and Tryptone provide the nitrogen, vitamins and amino acids in CLED Agar. L-Cystine is added as a growth supplement for cystine-dependent coliforms. Lactose is included as a carbon source. Organisms capable of fermenting lactose will lower the pH and change the color of the medium from green to yellow. Brom Thymol Blue is used as a pH indicator. Bacto Agar is used as a solidifying agent.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige with slight green tint, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water upon boiling. Solution is bluish-green, very slightly opalescent without precipitate.
Prepared Medium:	Bluish-green, very slightly opalescent without precipitate.
Reaction of 3.6% Solution:	pH 7.3 ± 0.2

Cultural Response

Prepare CLED Agar per label directions. Inoculate by spread plate technique and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	100-1,000	good	yellow
Proteus vulgaris	8427	100-1,000	good, swarming inhibited	blue to blue-green
Staphylococcus aureus	25923*	100-1,000	good	yellow

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

Formula

CLED Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	4 g
Bacto Tryptone	4 g
L-Cystine	0.128 g
Bacto Lactose	10 g
Bacto Agar	15 g
Bacto Brom Thymol Blue	0.02 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

CLED Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy. For best results, inoculate medium with specimen as soon as possible.

Test Procedure

For a complete discussion on collection and processing of urine cultures refer to appropriate references.[5,6,7]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. CLED Agar is basically non-selective. However, due to electrolyte exclusion, the growth of *Shigella* species is usually inhibited.[4]

References

1. **Sandys, G. H.** 1960. A new method of preventing swarming of *Proteus* spp. with a description of a new medium suitable for use in routine laboratory practice. J. Med. Lab. Technol. **17**:224.
2. **Mackey, J. P., and G. H. Sandys.** 1965. Laboratory diagnosis of infections of the urinary tract in general practice by means of a dip-inoculum transport medium. Br. Med. J. **2**:1286.
3. **Mackey, J. P., and G. H. Sandys.** 1966. Diagnosis of urinary tract infections. Br. Med. J. **1**:1173.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

CLED Agar	500 g	0971-17
	10 kg	0971-08

Bacto® Campylobacter Agar Base · Bacto Campylobacter Agar Kit Blaser · Bacto Campylobacter Agar Kit Skirrow

Intended Use

Bacto Campylobacter Agar Base is used with blood and Bacto Campylobacter Antimicrobic Supplement B (Blaser), Bacto Campylobacter Antimicrobic Supplement S (Skirrow) or other antibiotics in isolating and cultivating *Campylobacter.*

Also Known As

Campylobacter Agar Kit Skirrow is used to prepare Campylobacter Agar, Skirrow's or Skirrow's Campylobacter Agar.

Campylobacter Agar Kit Blaser is used to prepare Campylobacter Agar, Blaser's or Blaser's Campylobacter Agar.

Summary and Explanation

The genus *Campylobacter* was proposed in 1963 for *Vibrio fetus*, a species not exhibiting true characteristics of *Vibrionaceae*.[1] In 1977, Skirrow succeeded in isolating *C. jejuni* from fecal samples. Skirrow used a selective medium, incubated at 42°C in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. Skirrow confirmed this organism as a major etiologic agent of human enteritis,[1] an infection acquired through ingestion of water or food contaminated with the microorganism.

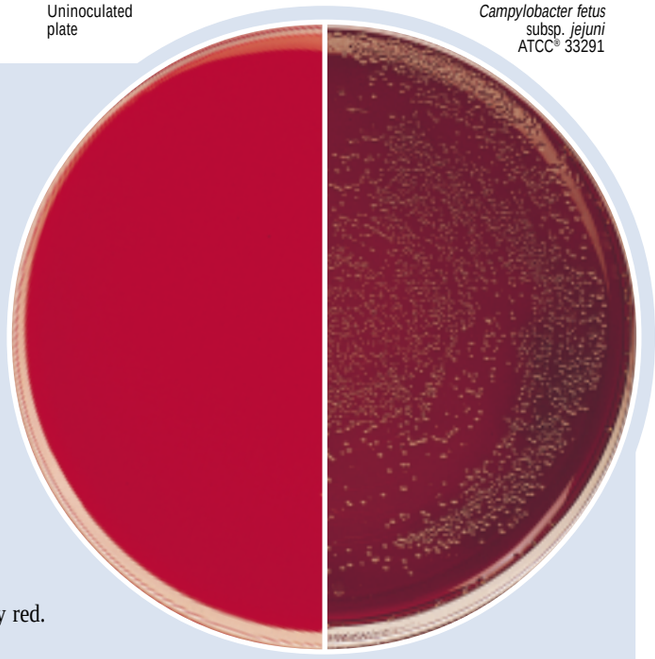
The Skirrow formulation includes blood agar supplemented with vancomycin, polymyxin B and trimethoprim for the selective isolation of *C. fetus* subsp. *jejuni*.[2] Blaser et al. further Incorporated cephalothin and amphotericin B to improve inhibition of normal enteric flora.

In 1983, spiral-shaped organisms resembling campylobacteria were isolated from the human stomach. The discovery sparked renewed interest in the etiology of human type B gastritis.[1] After genetic analysis, the genus *Helicobacter* was created and most attention focused on *H. pylori*. Specimens of gastric biopsies, brushings, or aspirates are used for the detection of *H. pylori*. Chocolate agar and brain heart infusion or brucella agar, enriched with 5 to 7% horse or rabbit blood, will support the growth of *H. pylori*.[1]

The Skirrow formulation is recommended for clinical specimens.[1] Campylobacter Agar Base is specified for food testing in Standard Methods.[3,4]

Principles of the Procedure

Campylobacter Agar Base is a nutritionally rich medium based on Blood Agar Base No. 2, rather than on Brucella Agar, to support more luxuriant *Campylobacter* growth because Trimethoprim is more active in Blood Agar Base No. 2. Supplementation of the base with antimicrobial agents as described by Skirrow[2] and Blaser et al.[5,6] provides for markedly reduced growth of normal enteric bacteria and improved recovery of *C. fetus* supsp. *jejuni* from fecal specimens. Growth of fungi is markedly to completely inhibited with Campylobacter Antimicrobic Supplement B due to the presence of amphotericin B.



User Quality Control

Identity Specifications

Campylobacter Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in distilled or deionized water upon boiling; medium to dark amber, clear to slightly opalescent.
Prepared Medium:	Without blood: medium to dark amber, very slightly to slightly opalescent without significant precipitate.
	With 10% sheep blood: cherry red, opaque.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

Campylobacter Antimicrobic Supplement B

Lyophilized Appearance: Bright medium yellow cake or powder.

Rehydrated Appearance: Yellow suspension.

Prepared Medium: Blaser formulation: opaque, medium cherry red.

Campylobacter Antimicrobic Supplement S

Lyophilized Appearance: White cake or powder.

Rehydrated Appearance: Colorless, clear.

Prepared Medium: Skirrow formulation: translucent, dark red.

Cultural Response

Prepare Campylobacter Agar Blaser or Skirrow per label directions. Inoculate and incubate at 42°C for 40-48 hours.

ORGANISM	ATCC®	CFU	GROWTH	APPEARANCE
Campylobacter fetus subsp. *jejuni*	33291	100-1,000	good	non-hemolytic, mucoid, gray colonies
Candida albicans	10231†	2,000-10,000	marked to complete inhibition	
Enterococcus faecalis	33186	2,000-10,000	marked to complete inhibition	
Escherichia coli	25922*	2,000-10,000	marked to complete inhibition	

†This organism is tested on Campylobacter Agar Blaser, only.

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.

Formula

Campylobacter Agar Base

Formula Per Liter

Bacto Proteose Peptone No. 3	15 g
Liver Digest	2.5 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g
Bacto Agar	12 g

Campylobacter Antimicrobic Supplement B

Ingredients per vial	10 ml vial	5 ml vial
Vancomycin	10 mg	5 mg
Polymyxin B	2,500 units	1,250 units
Trimethoprim	5 mg	2.5 mg
Cephalothin	15 mg	7.5 mg
Amphotericin B	3 mg	1 mg

Campylobacter Antimicrobic Supplement S

Ingredients per vial	10 ml vial	5 ml vial
Vancomycin	10 mg	5 mg
Polymyxin B	2,500 units	1,250 units
Trimethoprim	5 mg	2.5 mg

Precautions

1. For Laboratory Use.
2. **Campylobacter Antimicrobic Supplement B**

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE SENSITIZATION BY INHALATION AND SKIN CONTACT. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. 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, Ears, Bone Marrow.

   Campylobacter Antimicrobic Supplement S

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE SENSITIZATION BY INHALATION AND SKIN CONTACT. 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: Kidneys, Ears, Bone Marrow.

   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 lyophilized and rehydrated Campylobacter Antimicrobic Supplements B and S at 2-8°C. Use the rehydrated supplement within 24 hours.

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

Campylobacter Agar Base
Campylobacter Antimicrobic Supplement B
Campylobacter Antimicrobic Supplement S

Materials Required But Not Provided

Specimen collection containers or sterile rectal swabs
Microaerophilic environment system
Bunsen burner or incinerator
Sterile defibrinated blood or sterile lysed horse blood
Inoculating loops
Incubator (42°C)
Sterile Petri dishes

Method of Preparation

Campylobacter Agar Base:

1. Suspend 39.5 grams of Campylobacter Agar Base in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C. Aseptically add 5-7% sterile lysed horse blood (final concentration) or 10% sterile defibrinated sheep blood (final concentration).
5. Aseptically add 1% rehydrated Campylobacter Antimicrobic Supplement B or Campylobacter Antimicrobic Supplement S (10 ml per liter or 5 ml per 500 ml of basal medium). Mix well.
6. Dispense 20 ml amounts into 90 mm Petri dishes.

Campylobacter Antimicrobic Supplement B
Campylobacter Antimicrobic Supplement S

1. Aseptically rehydrate the lyophilized supplement with 5 or 10 ml of sterile distilled or deionized water, depending on label directions.
2. Invert the vial gently several times to dissolve the powder. Use within 24 hours of rehydration.

Specimen Collection and Preparation

Fecal specimens should be collected in sterile containers or with a sterile rectal swab and transported immediately to the laboratory for processing. If the specimen cannot be inoculated onto appropriate media within four hours after collection, the specimen should be maintained or transported in Cary-Blair Transport Medium.[1]

Test Procedure

1. Inoculate the specimen directly onto the surface of the prepared Campylobacter Agar plate and streak for isolation.
2. Incubate at 42°C under a microaerophilic atmosphere containing 5-6% oxygen and 3-10% carbon dioxide. Consult appropriate references for specific information on establishing a microaerophilic environment.[1,3,7]

Results

The colonies of *Campylobacter* species appear as non-hemolytic, flat and gray with an irregular edge or raised and round with a mucoid appearance. Some strains may appear tan or slightly pink. Swarming

or spreading may be observed on moist surfaces. Growth of normal enteric bacteria is markedly to completely inhibited. Growth of fungi is markedly to completely inhibited on Campylobacter Agar Blaser. Colonies are selected for further biochemical characterization.

Identification is based on a positive oxidase reaction and characteristic darting motility in a wet mount.[1] For further differentiation into species and biotypes, test for catalase activity, urease, hydrogen sulfide production, nitrate reduction, hippurate, indoxyl acetate, DNA hydrolysis and susceptibility to cephalothin and nalidixic acid.[1]

Limitations of the Procedure

1. Campylobacter Agar prepared with either Campylobacter Antimicrobic Supplement S or Campylobacter Antimicrobic Supplement B is selective primarily for *Campylobacter* species. Biochemical testing using a pure culture is necessary for complete identification. Consult appropriate references for further information.[1,3,7]
2. Growth of *Campylobacter fetus* subsp. *intestinalis* may be dramatically inhibited on Campylobacter Agar Blaser due to the presence of cephalothin. The use of Campylobacter Agar Skirrow and incubation at 35°C is suggested when isolating this organism from mixed populations.
3. Some strains of *C. fetus* subsp. *jejuni* may be encountered that fail to grow or grow poorly on prepared Campylobacter Agar.
4. Some strains of normal enteric organisms may be encountered that are not inhibited or only partially inhibited on Campylobacter Agar.

References

1. **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.
2. **Skirrow, M. D**. 1977. *Campylobacter* enteritis: A “new” disease. Br. Med. J. **2**:9-11.
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.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Blaser, M. J., V. Berkowitz, F. M. LaForce, J. Cravens, L. B. Reller, and W. L. Wang**. 1979. *Campylobacter* enteritis: clinical and epidemiologic features. Ann. Intern. Med. **91**:179-185.
6. **Blaser, M. J., J. Cravens, B. W. Powers, and W. L. Wang**. 1978. *Campylobacter* enteritis associated with canine infection. Lancet **(ii)**:979-980.
7. **Koneman E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, W. C. Winn.** 1983. Color atlas and textbook of diagnostic microbiology, 5th ed. J. B. Lippencott-Raven Publishers. Washington, D.C.

Packaging

Campylobacter Agar Base	2 kg	1820-07
Campylobacter Agar Kit Blaser		3279-32
To prepare: **6 x 1 liter**		
Campylobacter Agar Base	6 x 39.5 grams	
Campylobacter Antimicrobic Supplement B	6 x 10 ml	
Campylobacter Agar Kit Blaser		3279-40
To prepare: **6 x 500 ml**		
Campylobacter Agar Base	6 x 19.75 grams	
Campylobacter Antimicrobic Supplement B	6 x 5 ml	
Campylobacter Agar Kit Skirrow		3280-32
To prepare: **6 x 1 liter**		
Campylobacter Agar Base	6 x 39.5 grams	
Campylobacter Antimicrobic Supplement S	6 x 10 ml	
Campylobacter Agar Kit Skirrow		3280-40
To prepare: **6 x 500 ml**		
Campylobacter Agar Base	6 x 19.75 grams	
Campylobacter Antimicrobic Supplement S	6 x 5 ml	

Bacto® Candida BCG Agar Base

Intended Use

Bacto Candida BCG Agar Base is used with added neomycin in isolating and differentiating *Candida* from primary specimens.

Also Known As

Candida BCG Agar Base is an abbreviation for Candida Brom Cresol Green Agar Base.

Summary and Explanation

Candida BCG Agar Base is prepared according to the formulation of Harold and Snyder.[1] Candida BCG Agar Base was developed after a study demonstrated triphenyltetrazolium chloride (TTC) employed in Pagano Levin medium retarded the growth of some *Candida* species. Harold and Snyder[1] used brom cresol green as the indicator, which is nontoxic to *Candida* species. This medium is primarily used for demonstrating morphological and biochemical reactions characterizing the different *Candida* species for clinical diagnosis.

Candidiasis is the most frequently encountered opportunistic fungal infection.[2] It is caused by a variety of species of *Candida*, with *Candida albicans* being the most frequent etiological agent, followed by *Candida tropicalis* and *Candida* (*Torulopsis*) *glabrata*.[2] *Candida* species can be present in clinical specimens as a result of environmental contamination, colonization, or actual disease process.[3]

Principle of the Procedure

Bacto Peptone provides the nitrogen and amino acids in Candida BCG Agar Base. Yeast Extract is the vitamin source. The high concentration of Dextrose provides carbon as an energy source in this formula. Bacto Agar is the solidifying agent. Brom cresol green is the pH indicator, and acid production changes the medium from blue-green to yellow. Due to pH changes, specific color patterns appear in the base and surface of colonies for differentiation of *Candida* species.

Neomycin is added to the medium in a concentration of 500 µg/ml. Neomycin and brom cresol green act as selective agents to inhibit bacteria in Candida BCG Agar Base.[4]

Formula

Candida BCG Agar Base

Formula Per Liter

Bacto Peptone	10 g
Bacto Yeast Extract	1 g
Bacto Dextrose	40 g
Bacto Agar	15 g
Brom Cresol Green	0.02 g

Final pH 6.1 ± 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 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

Candida BCG Agar Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (30°C)
Waterbath (45-55°C)
Neomycin (500 µg/ml)
Sterile Petri dishes

Method of Preparation

1. Suspend 66 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool the medium to 50-55°C. Add sterile neomycin (500 µg/ml). Mix well.

Specimen Collection and Preparation

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

Test Procedure

Refer to the scheme for yeast identification.[3] For a complete discussion on the isolation and identification of *Candida* species refer to the procedures described in the appropriate references.[2,3,5]

Results

Identification of *Candida* species on the basis of colony morphology on Candida BCG Agar follows:

***C. albicans*:** Colonies appear as blunt cones 4.5-5.5 mm diameter with smooth edges and surfaces; coarse feathery growths may arise from the center of the colony base to penetrate the medium. The color of

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige to blue-green, free-flowing, homogeneous.
Solution:	6.6% solution, soluble in distilled or deionized water on boiling, blue-green, slightly opalescent to opalescent, may have a precipitate.
Prepared Medium:	Blue-green to greenish blue, slightly opalescent to opalescent; may have a precipitate.
Reaction of 6.6% Solution at 25°C:	pH 6.1 ± 0.1

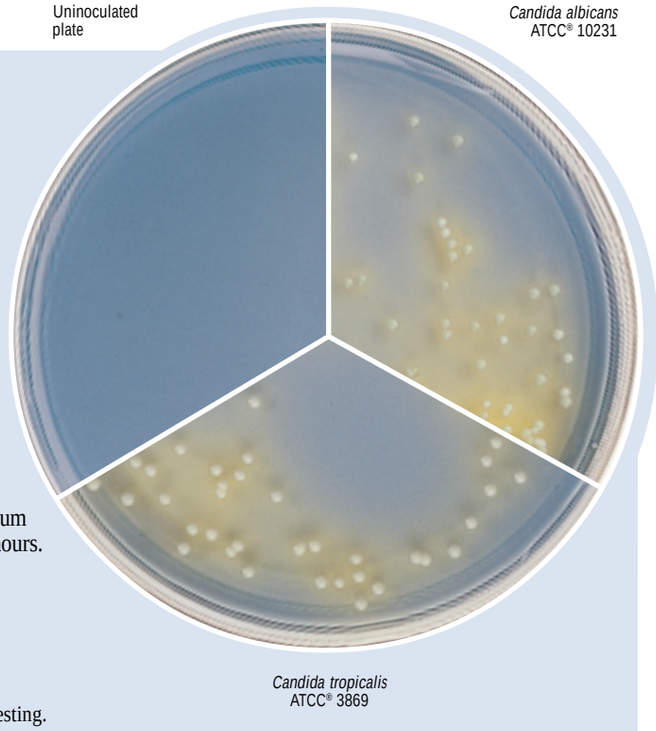
Cultural Response

Prepare Candida BCG Agar Base per label directions. Inoculate medium using the streak plate technique, and incubate at 30 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR OF MEDIUM
Candida albicans	10231	100-1,000	good	yellow
Candida tropicalis	3869	100-1,000	good	yellow
Escherichia coli	25922*	1,000-2,000	inhibited	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.



the base and surface of the colonies is yellowish to bluish green with the intensity diminishing from a gray green center spot to paleness at the edge, although some strains may show a distinct green outer ring.

***C. stellatoidea*:** Colonies appear convex 4.0-5.0 mm in diameter, with smooth edges and smooth to irregular surfaces; there is a fine central basal feathery growth penetrating the medium. The color of both base and surface of colonies is yellow to green, the intensity of which may or may not diminish from center to border but is usually light.

***C. tropicalis*:** Colonies appear convex or as low cones 4.5-5.0 mm in diameter with smooth to undulate edges, and smooth to granular or ridged surfaces; deeply stained feathery growth arises from several points in the base of the colony to form an effusive cloud. The color of the submerged growth is normally an intense blue green compared with that of the base which is much lighter; the surface is uniformly pale and may be yellowish green to green, reflecting a lower pH than observed of the base.

***C. pseudotropicalis*:** Colonies appear convex, 4.5-5.5 mm in diameter with undulate to smooth edges, and smooth surfaces; occasionally the surface is membranous but all colonies are shiny in appearance, and there is feathering growth emerging from several points in the base of the colony. The color of a large central area in the base of the colony is a medium green, which diminishes in intensity toward the edge; a similar distribution of color occurs on the surface, but this green is bright in hue and is never grayed as it is with *C. tropicalis*.

***C. krusei*:** Colonies appear as low cones 4.5-5.0 m in diameter with pseudohyphal edges, which may be weakly contractile or spreading, and have dull surfaces. There is abundant lightly colored growth penetrating the medium from the base of the colony. The base of the colony is a medium blue green in the center diminishing in intensity to paleness at the edge; the surface is usually a light green to yellow green without much concentration in any part.

***C. parapsilosis*:** Colonies appear as convex to low cones 3.5-4.5 mm in diameter with smooth or slightly spreading edges, but vary from smooth to granular or rough surfaces; there is no submerged growth. The color for both base and surface of the colony is blue green over much of the colony, being more intense in the base than the surface which is modified by a thin grayish film of cells; the intensity in color fades abruptly leaving a broad pale edge.

***C. guilliermondii*:** Colonies appear as low cones 4.0-5.0 mm in diameter with very smooth edges and highly glossy surfaces; there maybe a weak, fine feathered submerged growth. Both base and surface of the colony tend to have blue centers of medium intensity fading into a pale edge; however the surface may be blue green with the central third lightened with gray.

***C. glabrata*:** Colonies are smooth and convex, 4.6-5.0 mm diameter; the surface color pattern is pale green in the center which becomes medium green at the edge, and the base has the same color pattern but of less intensity.

Limitations of the Procedure

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

References

1. **Harold, W., and M. Snyder.** 1968. Personal Communication.
2. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
3. **Warren, N. G., and K. C. Hazen.** 1995. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 723-737. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 136-137. Williams & Wilkins, Baltimore, MD.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Packaging

Candida BCG Agar Base	500 g	0835-17

Bacto® Candida Isolation Agar

Intended Use

Bacto Candida Isolation Agar is used for isolating and differentiating *Candida albicans*.

Summary and Explanation

Candida Isolation Agar is a nutritionally rich medium that supports growth of many yeasts and molds and is differential for *Candida albicans*. Candida Isolation Agar was developed using a modification of YM Agar as described by Fung and Liang.[1] Goldschmidt demonstrated that YM Agar with Aniline Blue WS could be used to identify *C. albicans* in clinical samples with high accuracy and predictability.[2] Aniline Blue is metabolized by *C. albicans* to produce a fluorescent moiety that can be detected under long wave UV light.[2]

Principles of the Procedure

Yeast Extract provides nitrogen, carbon, vitamins and cofactors. Malt Extract provides carbon, protein and nutrients. Bacto Peptone provides additional carbon and nitrogen. Dextrose is an energy source. Aniline Blue is a fluorescent indicator. Bacto Agar is a solidifying agent.

Formula

Candida Isolation Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Bacto Peptone	5 g
Bacto Dextrose	10 g
Bacto Agar	20 g
Aniline Blue	0.1 g

Final pH 6.2 ± 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 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

Candida Isolation Agar

Materials Required but not Provided

Glassware

Autoclave

Method of Preparation

1. Suspend 41.1 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. Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory according to recommended guidelines.[3,4]

Test Procedure

1. Process each specimen as appropriate for that specimen and inoculate directly onto the surface of the medium. Streak for isolation.
2. Incubate plates aerobically at 30°C for 18-72 hours.
3. Examine plates for growth after 18-72 hours of incubation.

Results

Colonies of *C. albicans* fluoresce yellow-green under long wave UV light following incubation at 30°C for 18-24 hours. Non-*C. albicans* isolates do not fluoresce.

Limitations of the Procedure

1. Strains of *Candida albicans* have been reported that are false negative for fluorescence on this medium.[2]
2. Strains of *C. parapsilosis*, *C. krusei*, and *C. pulcherrima* that fluoresce on this medium may be encountered.[2] These strains may be distinguished from *C. albicans* based on germ tube formation in serum.[2,5]
3. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Fung, D. Y. C., and C. Liang.** 1988. A new fluorescent agar for the isolation of *Candida albicans*. Bull. Inf. Lab. Serv. Vet. (France) **29/30**:1-2.
2. **Goldschmidt, M. C., D. Y. C. Fung, R. Grant, J. White, and T. Brown.** 1991. New aniline blue dye medium for rapid identification and isolation of *Candida albicans*. J. Clin. Micro. **29**:1095-1099.
3. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection and handling, p. 19- 32. *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. **Splittstoesser, D. F., and C. Vanderzant (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **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

Candida Isolation Agar	500 g	0507-17

User Quality Control

Identity Specifications

Dehydrated
Medium Appearance: Beige, free-flowing, homogeneous.

Solution:	4.1% solution, soluble in distilled or deionized water on boiling. Solution is medium blue, very slightly opalescent.
Prepared Plates:	Medium blue, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 6.2 ± 0.2

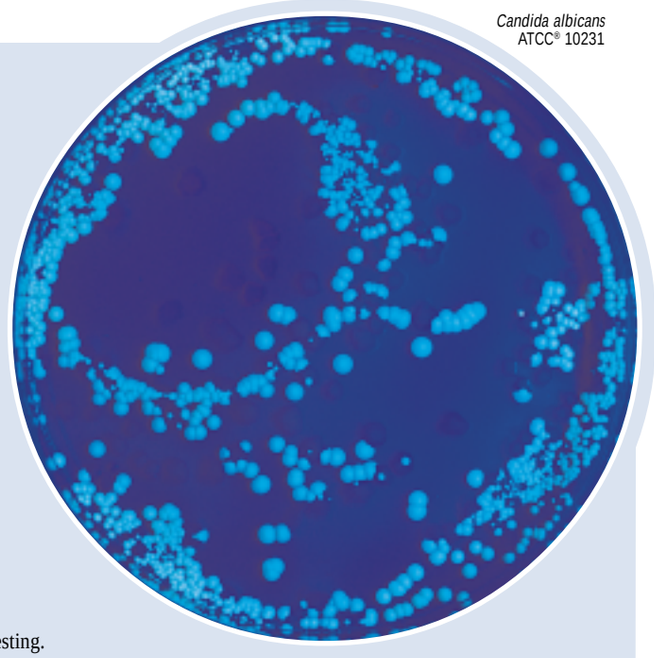
Cultural Response

Prepare Candida Isolation Agar per label instructions. Inoculate and incubate plates aerobically at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	FLUORESCENCE
Bacillus subtilis	6633	100-1,000	good	negative
Candida albicans	10231	100-1,000	good	positive
Escherichia coli	25922*	100-1,000	good	negative

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.



Candida albicans
ATCC® 10231

Bacto® Casamino Acids · Bacto Casamino Acids, Technical Bacto Vitamin Assay Casamino Acids

User Quality Control

Identity Specifications

Casamino Acids

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	1% solution-very light amber, clear solution.
	2% solution-Light amber, clear, soluble in distilled or deionized water upon slight heating.
Reaction of a 2% Solution at 25°C:	pH 5.8-6.65

Casamino Acids, Technical

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	1% solution, soluble in distilled or deionized water. Solution is colorless to very light amber and clear.
Reaction of 1% Solution at 25°C:	pH 5.0-7.5

Vitamin Assay Casamino Acids

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3% solution, soluble in distilled or deionized water on boiling. Very light to light amber, clear, may have a slight precipitate.
Reaction of 3% Solution at 25°C:	pH 6.5-8.5

Cultural Response

Casamino Acids and Casamino Acids, Technical

Prepare a 1% solution and adjust the pH to 7.2 ± 0.2. Inoculate tubes with the test organisms, and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Salmonella typhi	19430	100-1,000	good

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

Vitamin Assay Casamino Acids

Vitamin Assay Casamino Acids is prepared in various vitamin assay media to determine the vitamin content. It should not contain a vitamin content higher than 20% above the following values:

Vitamin B_{12}	0.2 nanograms/gram
Biotin	0.3 nanograms/gram
Folic Acid	3.3 nanograms/gram
Niacin	0.17 micrograms/gram
Pantothenate	0.04 micrograms/gram
Riboflavin	0.1 micrograms/gram
Thiamine	0.1 micrograms/gram

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

Intended Use

Bacto Casamino Acids is used in preparing microbiological culture media.

Bacto Casamino Acids, Technical is used in the preparation of microbiological culture media.

Bacto Vitamin Assay Casamino Acids is used in vitamin assay procedures.

Also Known As

Casamino Acids are also referred to as Casein Hydrolysate (Acid) or Casein Peptone, Acid Hydrolysate.

Summary and Explanation

Casamino Acids is acid hydrolyzed casein having low sodium chloride and iron concentrations. Casamino Acids is recommended for use in microbiological culture media that require a completely hydrolyzed protein as a nitrogen source. Casamino Acids is prepared according to the method described by Mueller and Miller[1] and Mueller and Johnson.[2]

Mueller[3] prepared diphtheria toxin in a medium containing a casein hydrolysate as the source of nitrogen. It was shown that the high sodium chloride content was the limiting factor in the amount of toxin that could be produced in this medium. Mueller and Miller[1] described a method to reduce the sodium chloride and iron content of the hydrolyzed casein. This hydrolyzed casein, supplemented with inorganic salts, growth factors, cystine, maltose and an optimum amount of iron, was used to prepare diphtheria toxin.[1,3] Casamino Acids duplicates this specially treated hydrolyzed casein.

In Casamino Acids, hydrolysis is carried out until all the nitrogen in the casein is converted to amino acids or other compounds of relative chemical simplicity. Casamino Acids is particularly well suited for nutritional studies, microbiological assays, and in the semi-synthetic medium for testing disinfectants.[4] Casamino Acids is also used in the preparation of tetanus toxins, and pertussis vaccines, and for sulfonamide inhibitor studies.[5]

Casamino Acids, Technical is acid hydrolyzed casein. The hydrolysis is carried out as in the preparation of Casamino Acids, but the sodium chloride and iron content of this product have not been decreased to the same extent. Casamino Acids, Technical is recommended for use in culture media where amino acid mixtures are required for a nitrogen source, and the sodium chloride content is slightly increased. It is particularly valuable in studying the growth requirements of bacteria.

Casamino Acids, Technical is prepared according to the method suggested by Mueller[1] for use in the preparation of diphtheria toxin. Mueller and Hinton[6] used Casamino Acids, Technical in a medium for primary isolation of gonococcus and meningococcus. Casamino Acids, Technical was used in agar-free media for the isolation of *Neisseria*, and in a tellurite medium for the isolation of *Corynebacterium*, described by Levin.[7] Wolf[8] used Casamino Acids, Technical in the preparation of a medium for the testing of disinfectants.

Vitamin Assay Casamino Acids is an acid digest of casein specially treated to markedly reduce or eliminate certain vitamins. It is

recommended for use in microbiological assay media and in studies of the growth requirements of microorganisms. Vitamin Assay Casamino Acids is commonly used as the amino acid source in early phases of nutrition work.[9] Sarett[10] used Vitamin Assay Casamino Acids as the acid hydrolyzed casein in his studies on *p*-aminobenzoic acid and *p*-teroylglutamic acid as growth factors for *Lactobacillus* species.

Several media containing Casamino Acids are specified in standard methods for multiple applications.[11,12,13]

Principles of the Procedure

Casamino Acids, Casamino Acids, Technical and Vitamin Assay Casamino Acids are acid hydrolyzed casein. Casein is milk protein, and a rich source of amino acid nitrogen. Casamino Acids, Casamino Acids, Technical and Vitamin Assay Casamino Acids provide nitrogen, vitamins, carbon and amino acids in microbiological culture media. Although Casamino Acids, Casamino Acids, Technical, and Vitamin Assay Casamino Acids are added to media primarily because of their organic nitrogen and growth factor components, their inorganic components also play a vital role.[14]

Formula

Casamino Acids is a dehydrated acid hydrolyzed casein in which Sodium Chloride and Iron are present in low concentrations permitting toxin production.

Casamino Acids, Technical is a dehydrated acid hydrolyzed casein. The Sodium Chloride and Iron content have not been reduced to same extent as Casamino Acids.

Vitamin Assay Casamino Acids is an acid hydrolyzed casein used to prepare media for microbiological assay of vitamins.

Typical Analysis

Physical Characteristics

Ash (%)	24.4	Loss on Drying (%)	4.5
Clarity, 1% Soln (NTU)	0.5	pH, 1% Soln	6.4
Filterability (g/cm^2)	2.9		

Nitrogen Content (%)

Total Nitrogen	10.5	AN/TN	83.8
Amino Nitrogen	8.8		

Amino Acids (%)

Alanine	3.26	Lysine	5.71
Arginine	2.20	Methionine	1.28
Aspartic Acid	4.76	Phenylalanine	2.11
Cystine	0.16	Proline	6.17
Glutamic Acid	15.30	Serine	2.19
Glycine	1.31	Threonine	2.41
Histidine	1.66	Tryptophan	<0.01
Isoleucine	3.34	Tyrosine	0.47
Leucine	5.47	Valine	4.30

Inorganics (%)

Calcium	<0.001	Phosphate	3.325
Chloride	7.400	Potassium	0.410
Cobalt	<0.001	Sodium	8.710
Copper	<0.001	Sulfate	0.045
Iron	<0.001	Sulfur	0.420
Lead	<0.001	Tin	<0.001
Magnesium	0.002	Zinc	<0.001
Manganese	<0.001		

Vitamins (µg/g)

Biotin	<0.1	PABA	<5.0
Choline (as Choline Chloride)	160.0	Pantothenic Acid	<0.1
Cyanocobalamin	<0.1	Pyridoxine	<0.1
Folic Acid	<0.1	Riboflavin	1.8
Inositol	<100.0	Thiamine	1.2
Nicotinic Acid	<20.0	Thymidine	<30.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	950
Salmonella	negative	Thermophile Count	25
Spore Count	390		

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Casamino Acids
Casamino Acids, Technical
Vitamin Assay Casamino Acids

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Casamino Acids, Casamino Acids, Technical or Vitamin Assay Casamino Acids in the formula of the medium being prepared. Add Casamino Acids, Casamino Acids, Technical or Vitamin Assay Casamino Acids 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 Casamino Acids, Casamino Acids, Technical or Vitamin Assay Casamino Acids.

Results

Refer to appropriate references and procedures for results.

References

1. **Mueller and Miller.** 1941. J. Immunol. **40**:21.
2. **Mueller and Johnson.** 1941. J. Immunol. **40**:33.
3. **Mueller**. 1939. J. Immunol. **37**:103.

4. **Klarman and Wright.** 1945. Soap and San. Chem. **21**:113.
5. **Straus, Dingle and Finland.** 1941. J. Immunol. **42**:331.
6. **Mueller and Hinton.** 1941. Proc. Soc. Exp. Biol. Med. **48**:330.
7. **Levin**. 1943. J. Bacteriol. **46**:233.
8. **Wolf**. 1945. J. Bacteriol. **49**:463.
9. **Nolan, R. A.** 1971. Amino acids and growth factors in vitamin-free casamino acids. Mycol. **63**:1231-1234.
10. **Sarett.** 1947. J. Biol. Chem. **171**:265.
11. **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.
12. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
13. **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.
14. **Nolan, R. A., and W. G. Nolan.** 1972. Elemental analysis of vitamin-free casamino acids. Appl. Microbiol. **24**:290-291.

Packaging

Casamino Acids	100 g	0230-15
	500 g	0230-17
	2 kg	0230-07
	10 kg	0230-08
Casamino Acids, Technical	500 g	0231-17
	10 kg	0231-08
Vitamin Assay Casamino Acids	100 g	0288-15
	500 g	0288-17

Bacto® Casein Digest

Intended Use

Bacto Casein Digest is used in preparing microbiological culture media.

Also Known As

Casein Digest is similar to N-Z-Amine A.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	1%, 2%, and 10% solutions, soluble in distilled or deionized water:
	1%-Light amber, clear;
	2%-Medium amber, clear;
	10%-Dark amber, clear, no significant precipitate.
Reaction of 1% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare NZM Broth per formula. 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 (HB101)	33694	100-1,000	good
Escherichia coli (JM107)	47014	100-1,000	good
Escherichia coli (DH5)	53868	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good
Streptomyces avermitilis	31267	100-1,000	good

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

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

Summary and Explanation

Casein Digest, an enzymatic digest of casein, was developed for use in molecular genetics media. This product is digested under conditions different from other enzymatic digests of casein, including Tryptone and Casitone.

Casein Digest is contained in the formulas of NZ media (NZCYM Broth, NZYM Broth and NZM Broth), which are used for cultivating recombinant strains of *Escherichia coli*. *E. coli* grows rapidly in these rich media because they provide amino acids, nucleotide precursors, vitamins and other metabolites that the cells would otherwise have to synthesize.[1] Consult appropriate references for recommended test procedures using NZ media.[1,2]

Principles of the Procedure

Casein Digest is a nitrogen and amino acid source for microbiological culture media. Casein is raw milk protein, a rich source of amino acid nitrogen.

Precautions

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

Storage

Store Casein Digest 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

Casein Digest

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Casein Digest in the formula of the medium being prepared. Add Casein Digest 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 Casein Digest.

Results

Refer to appropriate references and procedures for results.

References

1. **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.
2. **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

Casein Digest	500 g	0116-17

Bacto® Casitone

Intended Use

Bacto Casitone is used in preparing microbiological culture media.

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%-Light amber, clear, no precipitate.
- 2%-Light to medium amber, clear, may have a slight precipitate.
- 10%-Medium to dark amber, clear to very slightly opalescent, may have a precipitate.

Reaction of 1% Solution at 25°C: pH 6.8 - 7.4

Cultural Response

All solutions are prepared with the pH adjusted to 7.2 - 7.4.

TEST	SOLUTION	ORGANISM	ATCC®	RESULT	INOCULUM
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 Production	1%	*Salmonella typhi*	6539	positive	–
Toxicity	2%w/0.5% NaCl & 1.5% Agar	*Escherichia* coli	25922*	good growth	100-1,000
Toxicity	2%w/0.5% NaCl & 1.5% Agar	*Staphylococcus aureus*	25923*	good growth	100-1,000

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

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

Summary and Explanation

Casitone, is recommended for preparing media where an enzymatic hydrolyzed casein is desired. Casitone is used to support the growth of fastidious microorganisms. The high tryptophan content of Casitone makes it valuable for use in detecting indole production.

Dubos Broth and Dubos Oleic Agar media that support the growth of *Mycobacterium tuberculosis* contain Casitone. Media used for the enumeration of coliforms in water, m Endo Agar and m Endo Broth MF®, use Casitone as a nitrogen source. Several Thioglycollate media used for detecting microorganisms in normally sterile materials, include Casitone as a nitrogen and amino acid source.

Casitone is recommended for preparing media for sterility testing according to US Pharmacopeia XXIII (USP).[1] Several media containing Casitone are specified in standard methods[2,3,4,5] for multiple applications.

Principles of the Procedure

Casitone is a pancreatic digest of casein. Casein is the main protein of milk, and a rich source of amino acid nitrogen.

Precautions

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

Storage

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

Expiration Date

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

Typical Analysis

Physical Characteristics

Ash (%)	7.0	Loss on Drying (%)	3.7
Clarity, 1% Soln (NTU)	0.6	pH, 1% Soln	7.2
Filterability (g/cm^2)	1.7		

Carbohydrate (%)

Total	0.2

Nitrogen Content (%)

Total Nitrogen	13.3	AN/TN	35.3
Amino Nitrogen	4.7		

Amino Acids (%)

Alanine	3.01	Lysine	13.62
Arginine	3.76	Methionine	1.71
Aspartic Acid	6.61	Phenylalanine	4.02
Cystine	0.02	Proline	8.57
Glutamic Acid	20.03	Serine	4.82
Glycine	1.97	Threonine	3.74
Histidine	2.17	Tryptophan	0.14
Isoleucine	4.16	Tyrosine	2.09
Leucine	8.74	Valine	4.06

Inorganics (%)

Calcium	0.010	Phosphate	2.604
Chloride	0.110	Potassium	0.162
Cobalt	<0.001	Sodium	3.073
Copper	<0.001	Sulfate	0.339
Iron	0.003	Sulfur	0.676
Lead	<0.001	Tin	<0.001
Magnesium	0.019	Zinc	0.004
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	15.9
Choline (as Choline Chloride)	550.0	Pantothenic Acid	7.7
Cyanocobalamin	<0.1	Pyridoxine	1.3
Folic Acid	0.8	Riboflavin	0.4
Inositol	980.0	Thiamine	<0.1
Nicotinic Acid	20.3	Thymidine	342.9

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	1850
Salmonella	negative	Thermophile Count	100
Spore Count	300		

Procedure

Materials Provided

Casitone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Casitone in the formula of the medium being prepared. Add Casitone 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 Casitone.

Results

Refer to appropriate references and procedures for results.

References

1. **The United States Pharmacopeial Convention.** 1995. The United States Pharmacopeia, 23rd ed. Sterility test, p. 1686-1690. The United States Pharmacopeial Convention Inc., Rockville, MD.
2. **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.
3. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
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 examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.

Packaging

Casitone	100 g	0259-15
	500 g	0259-17
	10 kg	0259-08

Bacto® Casman Medium Base

Intended Use

Bacto Casman Medium Base is used with blood in isolating fastidious microorganisms under reduced oxygen tension.

Summary and Explanation

In 1947, Casman[1,2,3] described an infusion-free medium enriched with 5% blood for fastidious microorganisms incubated anaerobically. This medium replaced labor intensive formulas containing fresh meat infusion and unheated and heated blood.[1] Casman adjusted the medium after experiments revealed that nicotinamide disrupted the action of a blood enzyme that inactivates V factor (NAD).[2] Using unheated human blood in the formula, *Haemophilus influenzae* grew well and *Neisseria* was inhibited. The concentration of nicotinamide was lowered to support growth of *Neisseria* species.[2,3]

Casman Agar Base with rabbit blood can be used for the cultivation and maintenance of *Gardnerella vaginalis*.[4]

Principles of the Procedure

Proteose Peptone No.3, Tryptose and Beef Extract provide nitrogen, vitamins and amino acids. Nicotinamide enhances growth of *N. gonorrhoeae* and *H. influenzae* by impeding the removal of coenzyme (V factor) by nucleotidase from the enriched blood. The small amount of Dextrose is added to enhance growth of pathogenic cocci. Sodium chloride maintains the osmotic balance of the medium. Para-aminobenzoic acid is a preservative. Corn starch is added to ensure that any toxic metabolites produced are absorbed, to neutralize glucose inhibition of beta-hemolysis[4] and to enhance *growth of Neisseria* species. Agar Noble is a solidifying agent.

Formula

Casman Medium Base

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Nicotinamide	0.05 g
p-Aminobenzoic Acid	0.05 g
Bacto Dextrose	0.5 g
Corn Starch	1 g
Sodium Chloride	5 g
Agar Noble	14 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

Casman Medium Base

Materials Required But Not Provided

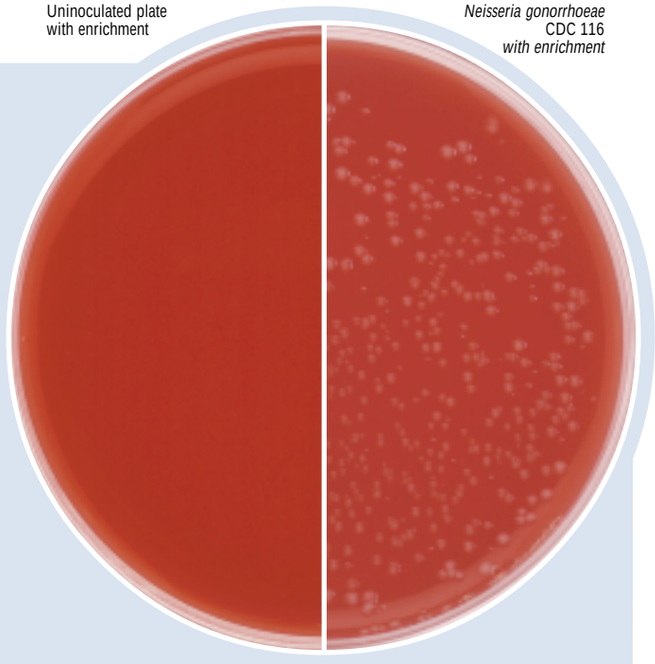
Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood
Sterile water-lysed blood

Method of Preparation

1. Suspend 43 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50°C.
4. Add 5% sterile blood and 0.15% sterile water-lysed blood solution (one part blood to three parts water). Omit water-lysed blood if sterile blood is partially lysed.
5. Dispense into sterile Petri dishes or as desired.

Specimen Collection and Preparation

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



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.3% solution, soluble in distilled or deionized water with frequent agitation on boiling. Light to medium amber with a ground glass appearance.
Prepared Medium:	Without blood, light to medium amber with a ground glass appearance. With 5% blood, cherry red opaque.
Reaction of 4.3% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Casman Medium Base per label directions, enrich with 5% sterile blood and 0.15% sterile water-lysed blood solution. Inoculateprepared medium and incubate at 35 ± 2°C under increased CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/BLOOD
Haemophilus influenzae	10211	100-1,000	good
Neisseria gonorrhoeae	CDC116	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

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

Test Procedure

For a complete discussion on the isolation and identification of fastidious microorganisms, refer to the procedures described in appropriate references.[5,6]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Nicotinamide in concentrations greater than 0.005% inhibits growth of some *N. gonorrhoeae* strains; however, only slight stimulation of growth of *H. influenzae* occurs with this amount.[1]
3. Hemolytic reactions of some strains have been shown to be affected by differences in animal blood.[6]
4. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[6]
5. Casman Medium Base is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[5,6]
6. Improper specimen collection, environment, temperature, CO_2 level, moisture and pH can adversely affect the growth and viability of the organism.

References

1. **Casman, E. P.** 1947. A noninfusion blood agar base for *Neisseriae*, *Pneumococci* and *Streptococci*. Am. J. Clin. Pathol. **27**:281.
2. **Casman, E. P.** 1942. J. Bacteriol. **43**:33.
3. **Casman, E. P.** 1947. J. Bacteriol. **53**:561.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 141-143. Williams & Wilkins, Baltimore, MD.
5. **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.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology. Washington, D.C.

Packaging

Casman Medium Base	500 g	0290-17

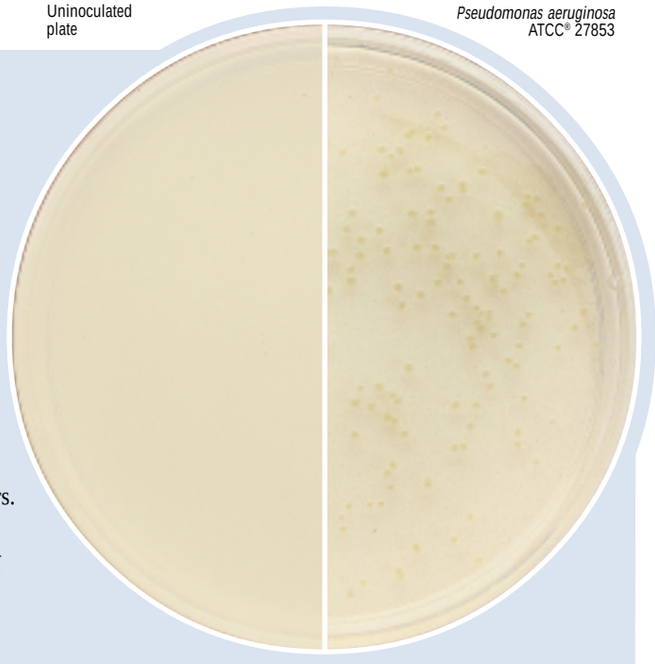
Bacto® Cetrimide Agar Base

Intended Use

Bacto Cetrimide Agar Base is used for isolating and cultivating *Pseudomonas aeruginosa*.

Also Known As

Cetrimide Agar Base is also referred to as Pseudosel™ Agar, Pseudomonas Selective Agar Base or Pseudomonas Selective Medium.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.53% solution with 1% glycerol, soluble on boiling in distilled or deionized water. Light amber, opalescent, with precipitate.
Prepared Medium:	Light amber, opalescent, with precipitate.
Reaction of 4.53% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Cetrimide Agar Base per label directions with 1% glycerol. Inoculate prepared medium and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU(approx.)	COLONY GROWTH	COLOR
Pseudomonas aeruginosa	27853*	1,000	good	yellow-green to blue
Escherichia coli	25922*	1,000-2,000	inhibited	–
Staphylococcus aureus	25923*	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.

Summary and Explanation

Pseudomonas aeruginosa has one of the broadest ranges of infectivity among pathogens, and is the most frequently isolated nonfermentative bacillus in clinical specimens.[3] It is a significant cause of burn and nosocomial infections.[4] The ability of *P. aeruginosa* strains to destroy tissue may be related to the production of various extracellular enzymes.[3] In addition, virulent strains produce an exotoxin A, which inhibits protein synthesis.[3]

Pseudomonas aeruginosa produces a number of water-soluble pigments, including the yellow-green or yellow-brown fluorescent pigment pyoverdin.[4] When pyoverdin combines with the blue water-soluble pigment pyocyanin, the bright green color characteristic of *P. aeruginosa* is created.[4] Fluorescent pigment-producing strains fluoresce under short-wave ultraviolet light, and are observed at 254 nm using a standard Wood's lamp.[3] Agar containing cetrimide has been used successfully to isolate *P. aeruginosa* from contaminated specimens.[5]

King, Ward and Raney[1] developed Medium A (Tech Agar) to enhance the production of pyocyanin in *Pseudomonas* species. Cetrimide Agar Base is prepared according to this formula with the addition of cetrimide.[1] Brown and Lowbury[2] used cetrimide in the Medium B formulation of King, Ward and Raney[1] to demonstrate the production of fluorescein in *P. aeruginosa*.

Cetrimide Agar Base is recommended in the examination of food[6] and in United States Pharmacopeia (USP XXIII) for use in Microbial Limit Tests.[7]

Principles of the Procedure

Bacto Peptone provides the nitrogen, vitamins and amino acids in Cetrimide Agar Base. Magnesium Chloride and Potassium Sulfate enhance the production of pyocyanin and fluorescein.[8] Cetrimide (cetyltrimethylammonium bromide) is the selective agent. Cetrimide acts as a quaternary ammonium cationic detergent causing nitrogen and phosphorous to be released from bacterial cells other than *P. aeruginosa*. Bacto Agar is the solidifying agent. Cetrimide Agar Base is supplemented with 1% Glycerol as a source of carbon.

Formula

Cetrimide Agar Base

Formula Per Liter

Bacto Peptone	20 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10 g
Cetrimide (Cetyltrimethylammonium Bromide)	0.3 g
Bacto Agar	13.6 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.

Procedure

Materials Provided

Cetrimide Agar Base

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 45.3 grams in 1 liter distilled or deionized water containing 10 ml of glycerol.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Dispense into sterile Petri dishes, or as desired.

Specimen Collection and Preparation

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

Test Procedure

For the isolation of *P. aeruginosa* plates of Cetrimide Agar Base may be inoculated by the streak method from nonselective medium or directly from the specimen. When plating directly from the specimen the inoculum level should be sufficiently high.

Results

Examine plates or tubes for the presence of characteristic blue, blue-green, yellow-green pigment. *Pseudomonas aeruginosa* typically produce both pyocyanin and fluorescein.

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. The type of peptone used in base may affect pigment production.[1,9]
3. No single medium can be depended upon to exhibit all pigment producing *P. aeruginosa* strains.
4. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescein production since this yellowing does not fluoresce.[1]
5. Some nonfermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.[1]
6. Studies of Lowbury and Collins[10] showed *Ps. aeruginosa* may lose its fluorescence under UV if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.
7. Further tests are necessary for definitive identification of *P. aeruginosa*.

References

1. **King, E. O., M. K. Ward, and E. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301.

2. **Brown, V.I., and E. J. L. Lowbury.** 1965. Use of an improved Cetrimide Agar Medium and of culture methods for Pseudomonas aeruginosa. J. Clin. Pathol. **18**:752.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
4. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.)., Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Robin, T., and J. M. Janda.** 1984. Enhanced recovery of *Pseudomonas aeruginosa* from diverse clinical specimens on a new selective agar. Diag. Microbiol. Infect. Dis. **2**:207.
6. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **The United States Pharmacopeia**. 1995. Microbiological Limits Tests, The United States pharmacopeia, 23rd ed. United States Pharmacopeial Convention, Rockville, MD.
8. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 146-149. Williams & Wilkins, Baltimore, MD.
9. **Goto, S., and S. Enomoto.** 1970. Nalidixic acid cetrimide agar: A new selective plating medium for the selective isolation of *Pseudomonas aeruginosa*. Jpn. J. Microbiol. **14**:65.
10. **Lowbury, E. J. L., and A. G. Collins.** 1955. The use of a new cetrimide product in a selective medium for *Pseudomonas aeruginosa*. J. Clin. Pathol. **8**:47.

Packaging

Cetrimide Agar Base	100 g	0854-15
	500 g	0854-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Chapman Stone Medium

Intended Use

Bacto Chapman Stone Medium is used for isolating and differentiating staphylococci based on mannitol fermentation and gelatinase activity.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous with a tendency to cake.
Solution:	20.2% solution, soluble in distilled or deionized water on boiling. Solution is light amber, opalescent with precipitation.
Prepared medium:	Light to medium amber, opalescent with a precipitate.
Reaction of 20.2% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Chapman Stone Medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours. Add Brom Cresol Purple indicator to determine mannitol fermentation (yellow = positive).

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HALO (Gelatinase)	MANNITOL FERMENTATION
Escherichia coli	25922*	100-1,000	inhibited	–	–
Staphylococcus aureus	25923*	100-1,000	good	+	+
Staphylococcus epidermidis	12228*	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.

Also Known As

Chapman Stone Medium conforms with Chapman Stone Agar.

Summary and Explanation

Chapman Stone Medium is prepared according to the formula described by Chapman.[1] It is similar to Staphylococcus Medium 110, previously described by Chapman,[2] except that the sodium chloride concentration is reduced to 5.5% and ammonium sulfate is included in the formulation. The inclusion of ammonium sulfate in the medium negates the need to add a reagent after growth has been obtained in order to detect gelatinase activity by Stone's method. Chapman Stone Medium is especially recommended for suspected food poisoning studies involving *Staphylococcus*.[3] It is selective, due to the relatively high salt content, and is differential due to pigmentation, mannitol fermentation, and the presence or absence of gelatin liquefaction.

Principles of the Procedure

Yeast Extract and Tryptone provide nitrogen, carbon, sulfur, vitamins, and trace nutrients essential for growth. Gelatin serves as a substrate for gelatinase activity. Ammonium Sulfate allows detection of gelatin hydrolysis. D-Mannitol is the fermentable carbohydrate. Sodium Chloride acts as a selective agent because most bacterial species are inhibited by the high salt content. Dipotassium Phosphate provides buffering capability. Bacto Agar is the solidifying agent.

Formula

Chapman Stone Medium

Formula Per Liter

Bacto Yeast Extract	2.5 g
Bacto Tryptone	10 g
Bacto Gelatin	30 g
Bacto D-Mannitol	10 g
Sodium Chloride	55 g
Ammonium Sulfate	75 g
Dipotassium Phosphate	5 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL IF SWALLOWED. (EC) 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. 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

Chapman Stone Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (30°C)
Sterile Petri dishes
Brom Cresol Purple

Method of Preparation

1. Suspend 20.2 grams in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 10 minutes. Omit sterilization if prepared medium is to be used within 12 hours.
4. Dispense as desired.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Streak a sample of the specimen onto the surface of the agar. Make several stabs into the medium along the streak.
2. Incubate, aerobically, at 30 ± 2°C for up to 48 hours.
3. Examine for growth and the presence or absence of clear zones around colonies.
4. To determine mannitol fermentation, add a few drops of Brom Cresol Purple to areas on the medium from which colonies have been removed. Any change in color of the indicator, compared with that of the uninoculated medium, indicates fermentation of mannitol.

Results

Mannitol fermentation: Positive = change in color of the indicator to yellow.

Gelatinase activity: Positive Stone reaction = formation of clear zones around the colonies.

Any mannitol-positive, yellow or orange colonies surrounded by a clear zone are presumptively identified as *Staphylococcus aureus*. White or nonpigmented colonies, with or without a clear zone, are probably *S. epidermidis*.

Limitations of the Procedure

1. Confirm the presumptive identification of pathogenic staphylococci with additional tests, such as coagulase activity.
2. Enterococci and/or Group D streptococci may exhibit growth on the medium and show slight mannitol fermentation. The colonies, however, are tiny and can easily be differentiated from staphylococci by Gram stain and the catalase test.[3]

References

1. **Chapman, G. H.** 1948. An improved Stone medium for the isolation and testing of food-poisoning staphylococci. Food Res. **13**:100-105.
2. **Chapman, G. H.** 1946. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation, and the Stone reaction. J. Bacteriol. **51**:409-410.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Chapman Stone Medium	500 g	0313-17
	10 kg	0313-08

Bacto® Charcoal Agar

Intended Use

Bacto Charcoal Agar is used for cultivating fastidious organisms, especially *Bordetella pertussis*, for vaccine production and stock culture maintenance.

Summary and Explanation

Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen.[1] The authors found this medium to be an efficient substitute for Bordet-Gengou Agar in the production of *B. pertussis* vaccines.

The genus *Bordetella* consists of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium*.[2] All *Bordetella* are

respiratory pathogens, residing on the mucous membranes of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder form of the disease.[3] *B. bronchiseptica* is an opportunistic human pathogen associated with both respiratory and non-respiratory infections, often occurring in patients having close contact with animals.[2] *B. bronchiseptica* has not been reported to cause pertussis. There have been no reports of recovery of *B. avium* from humans.[2]

Charcoal Agar supplemented with Horse Blood is used for the cultivation and isolation of *Haemophilus influenzae*.[4]

Principles of the Procedure

Infusion from Beef Heart and Bacto Peptone provide the nitrogen, carbon and amino acids in Charcoal Agar. Yeast Extract is a vitamin source. Sodium Chloride maintains osmotic balance. Bacto Agar is a solidifying agent. Soluble Starch absorbs toxic metabolites. Norit SG, charcoal, provides growth requirements and selective properties.

Formula

Charcoal Agar

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Peptone	10 g
Sodium Chloride	5 g
Bacto Soluble Starch	10 g
Bacto Yeast Extract	3.5 g
Norit SG	4 g
Bacto Agar	18 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

Charcoal Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 62.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Mix thoroughly during dispensing to uniformly distribute the charcoal.

User Quality Control

Identity Specifications

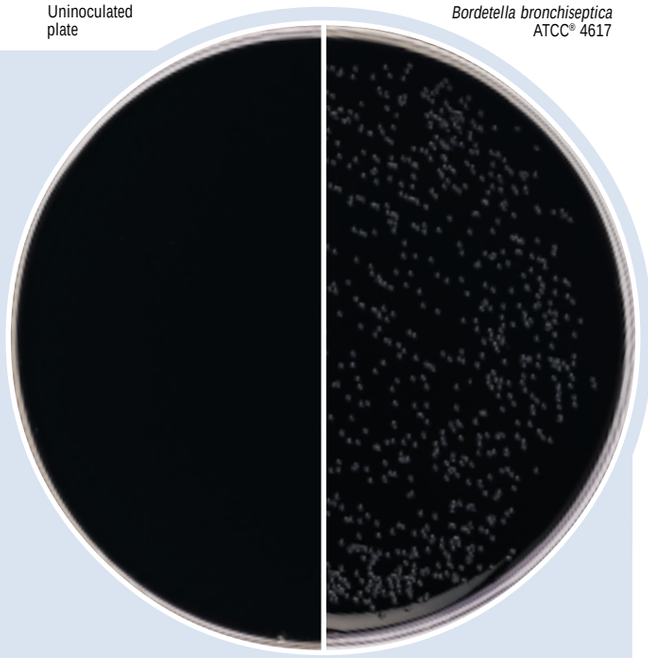
Dehydrated Appearance:	Gray, free-flowing, homogeneous.
Solution:	6.25% solution, soluble in distilled or deionized water on boiling; black, opaque with a precipitate.
Prepared Medium:	Black, opaque.
Reaction of 6.25% Solution at 25°C	pH 7.3 ± 0.2

Cultural Response

Prepare Charcoal Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bordetella bronchiseptica	4617	100-1,000	good
Bordetella parapertussis	15237	100-1,000	good
Bordetella pertussis	8467	100-1,000	good

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



Specimen Collection and Preparation

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

Test Procedure

For a complete discussion on the isolation and maintenance of fastidious microorganisms refer to the procedures described in appropriate references.[2,4,5]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.[4]

References

1. **Mishulow, L., L. S. Sharpe, and L. L. Cohen.** 1953. Beef-heart charcoal agar for the preparation of pertussis vaccines. Am. J. Public Health, **43**:1466.
2. **Marcon, M. J.** 1995. *Bordetella*, p. 566-573. *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. **Linneman, C. C., and E. B. Pery.** 1977. *Bordetella parapertussis*; recent experience and a review of the literature. Am. J. Dis. Child. **131**:560-563. *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. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1, p. 154-159. Williams & Wilkins, Baltimore, MD.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Charcoal Agar	500 g	0894-17

Bacto® Choline Assay Medium

Intended Use

Bacto Choline Assay Medium is used for determining choline concentration by the microbiological assay technique.

Summary and Explanation

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

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

Choline Assay Medium is a slight modification of the medium described by Horowitz and Beadle.[1] *Neurospora crassa* ATCC® 9277 is the test organism used in this microbiological assay.

User Quality Control

Identity Specifications

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	2.85% (single strength) and 5.7% (double strength) solution, soluble in distilled or deionized water upon boiling. Solution is colorless, clear, may have a slight precipitate.
Prepared Medium:	Colorless, clear, may have a slight precipitate.
Reaction of 2.85% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

Prepare Choline Assay Medium per label directions. Prepare a standard curve using choline at levels from 0 to 25 µg per 10 ml. The medium supports the growth of *Neurospora crassa* ATCC® 9277 when supplemented with choline chloride.

Principles of the Procedure

Choline Assay Medium is a choline-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *N. crassa* ATCC® 9277. The addition of choline standard in specified increasing concentrations gives a growth response by this organism that can be measured gravimetrically.

Formula

Choline Assay Medium

Formula Per Liter

Bacto Sucrose	40 g
Ammonium Nitrate	2 g
Biotin	10 µg
Potassium Sodium Tartrate	11.4 g
Monopotassium Phosphate	2 g
Magnesium Sulfate	1 g
Sodium Chloride	0.2 g
Calcium Chloride	0.2 g
Sodium Borate	700 µg
Ammonium Molybdate	500 µg
Ferrous Sulfate	1.1 mg
Cuprous Chloride	300 µg
Manganese Sulfate	110 µg
Zinc Sulfate	17.6 mg

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Choline Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Neurospora crassa* ATCC® 9277
Sterile 0.85% saline
Distilled or deionized water
Inoculating loop
Neurospora Culture Agar
Wire needle or glass rod
Paper towels
Vacuum oven
Porcelain spot plate
Scale

Method of Preparation

1. Suspend 5.7 grams in 100 ml of distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into flasks, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust flask volume 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

Remove 1 loop of spores from a 48-hour culture of *N. crassa* ATCC® 9277 grown on Neurospora Culture Agar and suspend it in 100 ml sterile saline. Add 1 drop of this spore suspension to each flask of medium. Incubate at 25-30°C for 3 days. At the end of the incubation period, steam the flask at 100°C for 5 minutes. Remove all the mycelium from the flask using a stiff wire needle or glass rod, press dry between paper towels, and roll into a small pellet. Dry the pellet at 100°C in a vacuum oven for 2 hours. (A glazed porcelain spot plate is convenient for handling the mycelium during drying and weighing.) Weigh to the nearest 0.5 mg. A standard curve is then constructed from the weights obtained, and the unknown determined by interpolation. In the assay for choline, 50 ml Erlenmeyer flasks containing a total volume of 10 ml each are used.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using choline at levels of 0.0, 2.5, 5, 10, 15, 20 and 25 µg per assay flask (10 ml). The most effective assay range using Choline Assay Medium is between 2.5 and 30 µg choline.

The concentration of choline required for the preparation of the standard curve may be prepared by dissolving 0.5 grams choline chloride in 1,000 ml distilled water. This is the stock solution (500 µg per ml). Dilute the stock solution by adding 1 ml to 99 ml distilled water. Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml of this diluted solution per flask. Prepare the stock solution fresh daily.

Results

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

Limitations of the Procedure

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

References

1. **Horowitz and Beadle.** 1943. J. Biol. Chem. **150**:325.

Packaging

Choline Assay Medium	100 g	0460-15

Bacto® Columbia Blood Agar Base · Bacto Columbia Blood Agar Base EH · Bacto Columbia Blood Agar Base No. 2

Intended Use

Bacto Columbia Blood Agar Base is used for cultivating fastidious microorganisms with or without the addition of blood.

Bacto Columbia Blood Agar Base EH is used with blood in isolating and cultivating fastidious microorganisms.

Bacto Columbia Blood Agar Base No. 2 is used with blood in isolating and cultivating fastidious microorganisms.

Also Known As

Blood Agar Base may be abbreviated as BAB.

Summary and Explanation

Columbia blood agar base media are typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining the hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, Columbia Blood Agar Base can be used as a general purpose medium.

Columbia Blood Agar Base was patterned after the Columbia Agar formulation described by Ellner *et al*. of Columbia University.[1] Columbia Blood Agar Base No. 2 and Columbia Blood Agar Base EH (Enhanced Hemolysis) are modifications of Columbia Blood Agar Base. Columbia Blood Agar Base No. 2 provides clearer hemolytic reactions with *Streptococcus* group A while Columbia Blood Agar Base EH provides dramatic, enhanced hemolysis.

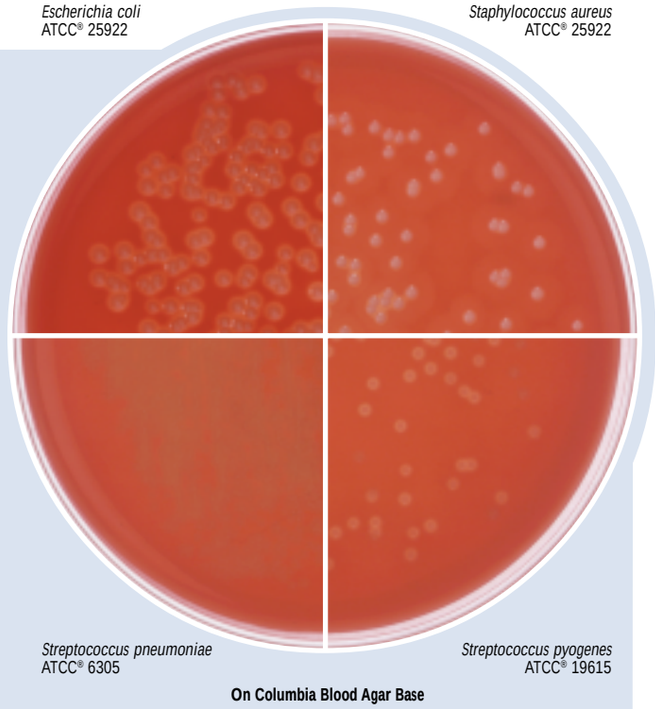
Columbia Blood Agar Base is specified in the Compendium of Methods for the Microbiological Examination of Foods.[2]

Principles of the Procedure

Columbia Blood Agar Base uses specially selected raw materials to support good growth of fastidious microorganisms. Two peptones, Pantone (a casein hydrolysate) and Bitone (an infusion peptone), provide nitrogen, carbon, amino acids and vitamins. Tryptic Digest of Beef Heart provides additional nitrogen and amino acids. Corn Starch, originally proposed by the authors of this medium, increases growth of *Neisseria* and enhances the hemolytic reactions of some streptococci.[1] Agar is a solidifying agent. Sodium Chloride maintains the osmotic balance of the medium.

Columbia Blood Agar Base No. 2 and Columbia Blood Agar Base EH are similar in composition to Columbia Blood Agar Base. However, different peptones are used to improve and enhance hemolysin production while minimizing antagonism or loss in activity of streptococcal hemolysins. Columbia Blood Agar Base No. 2 contains Bitone H while Columbia Blood Agar Base EH contains Bitone H Plus. Both formulations contain Pantone, Enzymatic Digest of Animal Tissue, Starch, Sodium Chloride and Agar.

Blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of ß-hemolytic streptococci.[3] Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.[4]



On Columbia Blood Agar Base

User Quality Control

Identity Specifications

Columbia Blood Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.4% solution, soluble in distilled or deionized water on boiling, light to medium amber, opalescent with a fine precipitate.
Prepared Medium:	Plain - light to medium amber, slightly opalescent to opalescent with a fine precipitate.
	With 5% sheep blood - cherry red, opaque.
Reaction of 4.4% Solution at 25°C:	pH 7.3 ± 0.2

Columbia Blood Agar Base No. 2

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in distilled or deionized water on boiling, light to medium amber, opalescent.
Prepared Medium:	With 5% sheep blood - cherry red, opaque.
Reaction of 3.9% Solution at 25°C:	pH 7.3 ± 0.2

continued on following page

Formula

Columbia Blood Agar Base

Formula Per Liter

Bacto Pantone	10 g
Bacto Bitone	10 g
Tryptic Digest of Beef Heart	3 g
Corn Starch	1 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Columbia Blood Agar Base EH

Formula Per Liter

Bacto Pantone	12 g
Bacto Bitone H Plus	6 g
Enzymatic Digest of Animal Tissue	3 g
Starch	1 g
Sodium Chloride	5 g
Agar	12 g

Final pH 7.3 ± 0.2 at 25°C

Columbia Blood Agar Base No. 2

Formula Per Liter

Bacto Pantone	12 g
Bacto Bitone H	6 g
Enzymatic Digest of Animal Tissue	3 g
Starch	1 g
Sodium Chloride	5 g
Agar	12 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

Columbia Blood Agar Base
Columbia Blood Agar Base EH
Columbia Blood Agar Base No. 2

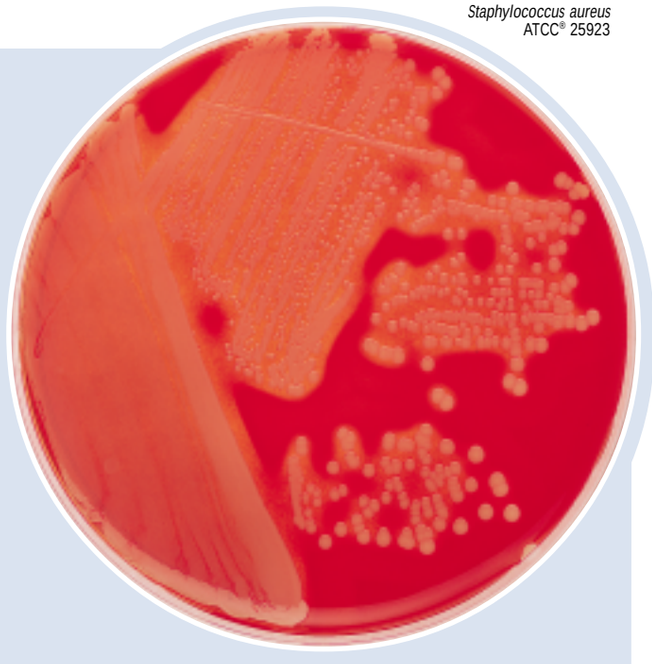
Materials Required But Not Provided

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

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Columbia Blood Agar Base - 44 grams;
   Columbia Blood Agar Base EH - 39 grams;
   Columbia Blood Agar Base No. 2 - 39 grams.

Staphylococcus aureus
ATCC® 25923



Columbia Blood Agar Base EH

User Quality Control cont.

Columbia Blood Agar Base EH

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in distilled or deionized water on boiling, light to medium amber, clear to slightly opalescent.
Prepared Medium:	With 5% sheep blood - medium to bright cherry red, opaque.
Reaction of 3.9% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare the medium with and without 5% sterile defibrinated sheep blood per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HEMOLYSIS
Escherichia coli	25922*	100-1,000	good	N/A
Staphylococcus aureus	25923*	100-1,000	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	alpha
Streptococcus pyogenes	19615*	100-1,000	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. Cool to 45-50°C.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

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

Test Procedure

1. Process each specimen as appropriate and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit ß-hemolytic streptococci beneath the agar surface. Subsurface growth will demonstrate the most reliable hemolytic reactions due to the activity of both oxygen-stable and oxygen-labile streptolysins.[4]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.

Results

1. Examine plates for growth and hemolytic reactions after 18-24 and 48 hours of incubation. Four types of hemolysis on blood agar media can be described:[5]
   a. Alpha-hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.
   b. Beta-hemolysis (ß) is the lysis of red blood cells, producing a clear zone surrounding the colony.
   c. Gamma-hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
   d. Alpha-prime-hemolysis (ά) is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Blood agar base media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on these media, biochemical and, if indicated, immunological testing using pure cultures is recommended for complete identification. Consult appropriate references for further information.
2. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
3. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are ß-hemolytic on horse, human and rabbit blood agar and α-hemolytic on sheep blood agar.[4]
4. Colonies of *Haemophilus haemolyticus* are ß-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of ß-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[6]
5. Atmosphere of incubation has been shown to influence hemolytic reactions of ß-hemolytic streptococci.[4] For optimal performance, incubate blood agar media under increased CO_2 or anaerobic conditions.

References

1. **Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi.** 1966. A new culture medium for medical bacteriology. Am. J. Clin. Pathol. **45:**502-504.
2. **Vanderzant, C. and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin Pathol. **17**:281-289.
4. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Isenberg, H. D. (ed).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
6. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.

Packaging

Columbia Blood Agar Base	500 g	0792-17
	2 kg	0792-07
	10 kg	0792-08
Columbia Blood Agar Base EH	500 g	0790-17
	2 kg	0790-07
	10 kg	0790-08
Columbia Blood Agar Base No. 2	500 g	0793-17
	2 kg	0793-07
	10 kg	0793-08

Bacto® Columbia Broth

Intended Use

Bacto Columbia Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

Columbia Broth is prepared according to the formulation described by Morello and Ellner.[1] In their study Columbia Broth, a medium developed for blood cultures, was superior to a commonly used general purpose broth for faster growth of *Staphylococcus aureus*, *E. coli* and streptococci (viridans and enterococcus groups). Columbia Broth, in the presence of CO_2 and supplemented with SPS, is an excellent blood culture medium.[2] In the study by Morello and Ellner,[1] the addition of sodium polyanetholsulfonate (SPS) in Columbia Broth was emphasized. SPS is an anticoagulant that inhibits serum bactericidal activity against many bacteria, inhibits phagocytosis, inactivates complement, and neutralizes lysozymes and the aminoglycoside class of antibiotics.[2]

Principles of the Procedure

Columbia Broth was formulated from Pantone and Bitone. Dextrose is added to the formula as a carbon energy source. The medium is

buffered with Tris. Corn Starch is omitted to reduce opalescence.[1] Cysteine is the reducing agent. Magnesium and Iron are added to facilitate organism growth.

Formula

Columbia Broth

Formula Per Liter

Bacto Pantone	10 g
Bacto Bitone	10 g
Tryptic Digest of Beef Heart	3 g
L-Cysteine Hydrochloride	0.1 g
Bacto Dextrose	2.5 g
Sodium Chloride	5 g
Magnesium Sulfate Anhydrous	0.1 g
Ferrous Sulfate	0.02 g
Sodium Carbonate	0.6 g
Tris (Hydroxymethyl) Aminomethane	0.83 g
Tris (Hydroxymethyl) Aminomethane HCl	2.86 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

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

Storage

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on warming. Solution is light amber, clear to very slightly opalescent, may have a slight amount of fine precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent, may have a slight amount of fine precipitate.
Reaction of 3.5% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Prepare Columbia Broth per label directions. Inoculate and incubate at 35 ± 2°C under appropriate conditions for 18-48 hours. Incubate *Bacteroides fragilis* anaerobically.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good
Bacteroides fragilis	25285	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 Bactrol Disks Technical Information.

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

Columbia Broth

Materials Required But Not Provided

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

Method of Preparation

1. Dissolve 35 grams in 1 liter distilled or deionized water.
2. Warm slightly if necessary to dissolve completely.
3. OPTIONAL: Sodium polyanetholesulfonate (SPS) may be added at this time with agitation to ensure a uniform solution. The culture medium should contain 0.025 to 0.05% SPS.
4. Distribute in suitable containers. Autoclave at 121°C for 15 minutes.
5. Allow to cool to room temperature before using.

Specimen Collection and Preparation

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

Test Procedure

Process clinical specimens from different body sites as described in Clinical Microbiology Procedures Handbook,[2] Manual of Clinical Microbiology[3] or according to laboratory procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. *Neisseria* spp. may be inhibited by SPS in Columbia Broth. The addition of 1.2% gelatin may counteract the inhibitory effect, but SPS may also inhibit other organisms.[2]
3. Opalescence in Columbia Broth cannot always be relied upon as evidence of bacterial growth in the bottle.
4. It is possible for significant numbers of viable bacteria to be present in an inoculated and incubated blood culture bottle without the usual signs of bacterial growth.

References

1. **Morello, J. A., and P. D. Ellner**. 1969. New medium for blood cultures. Appl. Microbiol. **17**:68-70.
2. **Isenberg, H. D. (ed).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.

3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Columbia Broth	500 g	0944-17
	2 kg	0944-07

Bacto® Columbia CNA Agar

Intended Use

Bacto Columbia CNA Agar is used with added blood in isolating gram-positive cocci.

Also Known As

Columbia CNA Agar is also referred to as Colistin Nalidixic Acid Agar.

Summary and Explanation

Ellner et al.[1] described Columbia CNA Agar, a variation of Columbia Blood Agar Base that is selective for gram-positive cocci. The antimicrobics colistin and nalidixic acid select for gram-positive organisms and fungi by suppressing gram-negative bacteria.[2] Columbia CNA Agar is recommended as a primary plating medium when culturing urine specimens.[3]

Principles of the Procedure

Columbia CNA Agar is Columbia Blood Agar Base supplemented with colistin (10 µg/ml) and nalidixic acid (15 µg/ml). The antimicrobial agents suppress growth of *Enterobacteriaceae* and *Pseudomonas* species while allowing yeasts, staphylococci, streptococci and enterococci to grow.[4] Certain gram-negative organisms, such as *Gardnerella vaginalis* and some *Bacteriodes* species, can grow very well on Columbia CNA Agar with blood.[4] Colistin disrupts the cell membrane of gram-negative organisms; it is particularly effective against *Pseudomonas* species.[2] Nalidixic acid blocks DNA replication in susceptible bacteria and acts against many gram-negative bacteria.[2]

Formula

Columbia CNA Agar

Formula Per Liter

Bacto Pantone	10 g
Bacto Bitone	10 g
Tryptic Digest of Beef Heart	3 g
Corn Starch	1 g
Sodium Chloride	5 g
Colistin Sulfate	10 mg
Nalidixic Acid	15 mg
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

User Quality Control

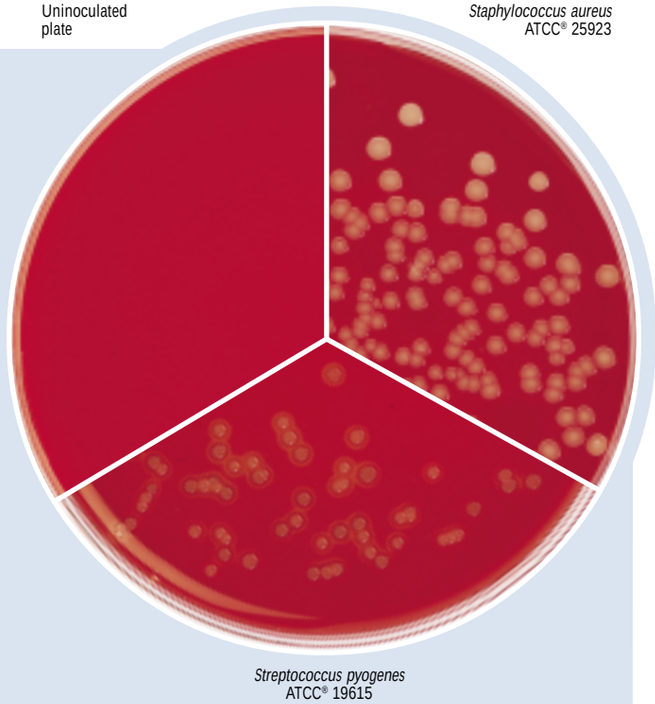
Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.4% solution, soluble in distilled or deionized water upon boiling, light to medium amber, slightly opalescent to opalescent with a fine precipitate.
Prepared Medium:	Without blood: light to medium amber, slightly opalescent to opalescent with a fine precipitate. With 5% sheep blood: cherry red, opaque.
Reaction of 4.4% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Columbia CNA Agar with and without 5% sheep blood per label directions. Inoculate both media and incubate at 35 ± 2°C for 18-24 hours under 10% CO_2.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o BLOOD	GROWTH w/BLOOD	HEMOLYSIS
Proteus mirabilis	12453	1,000-2,000	markedly inhibited	markedly inhibited	–
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



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

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

Precautions

1. For Laboratory Use.
2. 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 whenstored as directed. Do not use a product if it fails to meet specificationfor identity and performance.

Procedure

Materials Provided

Columbia CNA Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath
Sterile Petri dishes

Method of Preparation

1. Suspend 44 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121° C for 15 minutes. Avoid overheating.
4. Cool to 45-50°C.
5. Aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
6. Dispense into sterile Petri dishes or tubes as desired.

Specimen Collection and Preparation

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

Test Procedure

1. Inoculate specimens directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions due to the activity of both oxygen-stable and oxygen-labile streptolysins.[5]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.

Results

Examine plates for growth and hemolytic reactions after 18-24 and 48 hours incubation. Four different types of hemolysis on blood agar media can be described:[3]

a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish discolorization of the medium.
b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
d. Alpha-prime (ὰ)-hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit blood agar and alpha-hemolytic on sheep blood agar.[5]
3. Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies on beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[4]
4. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[5] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.
5. *Proteus* species occasionally grow on CNA Agar and may initially be confused with streptococci because of the small size of the colonies.[2]

References

1. **Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi.** 1966. A new culture medium for medical bacteriology. Am. J. Clin. Pathol. **45**:502-504.
2. **Estevez, E. G.** 1984. Bacteriologic plate media: review of mechanisms of action. Lab Med. **15**:258-262.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
4. **Baron, E. J., L. R. Peterson, and S.M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
5. **Ruoff, K. L.** 1995. Streptococcus, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Columbia CNA Agar	500 g	0867-17
	2 kg	0867-07

Bacto® Cooke Rose Bengal Agar Bacto Antimicrobic Vial A

Intended Use

Bacto Cooke Rose Bengal Agar is used with or without Bacto Antimicrobic Vial A in isolating fungi from environmental and food specimens.

Bacto Antimicrobic Vial A is used in preparing microbiological culture media.

Summary and Explanation

Cooke Rose Bengal Agar is a selective medium for the isolation of fungi prepared according to the formula of Cooke.[1] Selectivity of the medium may be increased by the addition of antibiotics.

A variety of materials and methods have been used to inhibit bacteria in an attempt to isolate fungi from mixed flora. Fungi are extremely successful organisms, as evidenced by their ubiquity in nature.[2] Waksman[3] described an acid medium consisting of peptone, dextrose, inorganic salts and agar for the isolation of fungi from soil. Cooke[1] used the Waksman[3] medium without adjustment to investigate the isolation of fungi from sewage. It was discovered that Soytone was particularly suitable for use in this medium and that the combination of chlortetracycline, or oxytetracycline, with rose bengal increased the selectivity of the medium.

Antimicrobic Vial A contains sterile, desiccated chlortetracycline (Aureomycin®). It was originally used in preparing DTM Agar described by Taplin, Azias, Rebell and Blank[4] for the isolation of dermatophytes. Antimicrobic Vial A is applicable for use in various media requiring this antibiotic. Cooke[1] preferred chlortetracycline in Cooke Rose Bengal Agar due to the increased stability of the antibiotic.

Principles of the Procedure

Soytone provides nitrogen, carbon and vitamins in Cooke Rose Bengal Agar. Dextrose is an energy source. Rose Bengal and chlortetracycline selectively inhibit bacterial growth and restrict the size and height of colonies of more rapidly growing molds. Monopotassium Phosphate provides buffering capability. Magnesium Sulfate is a source of divalent cations. Bacto Agar is a solidifying agent.

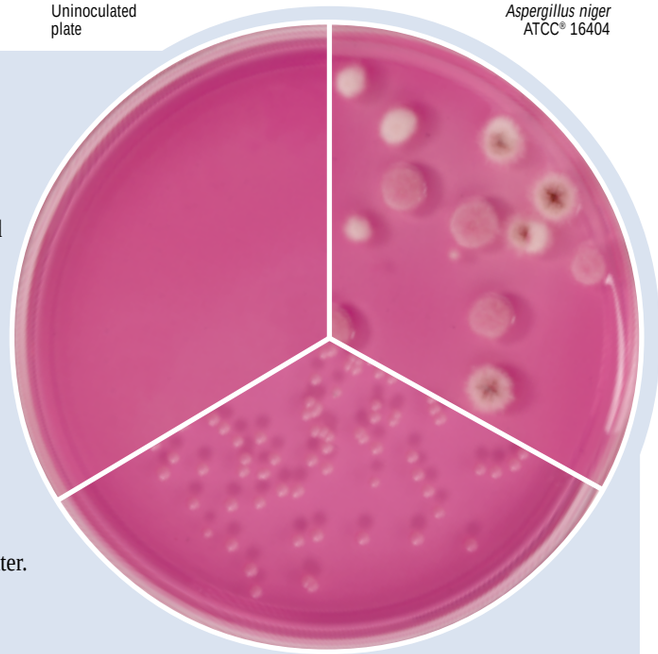
Formula

Cooke Rose Bengal Agar

Formula Per Liter

Bacto Soytone	5 g
Bacto Dextrose	10 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Bacto Agar	20 g
Rose Bengal	0.035 g

Final pH 6.0 ± 0.2 at 25°C



User Quality Control

Identity Specifications

Cooke Rose Bengal Agar

Dehydrated Appearance:	Pinkish tan, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling. Solution is pinkish red, very slightly to slightly opalescent without a significant precipitate.
Prepared Medium:	Deep pink, slightly opalescent without a precipitate.
Reaction of 3.6% Solution at 25°C:	pH 6.0 ± 0.2

Antimicrobic Vial A

Lyophilized Appearance:	Yellow cake or powder.
Rehydrated Appearance:	Yellow, clear solution.
Solution:	Soluble in 10 ml distilled or deionized water.
Microbial Limits Test:	Negative.
Potency (Cup-Plate Assay):	90-140% of labeled potency.

Cultural Response

Prepare Cooke Rose Bengal Agar with 35 µg per ml chlortetracycline (Antimicrobic Vial A) per label directions. Inoculate and incubate at 25-30°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	30-300	good
Candida albicans	26790	30-300	good
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition
Saccharomyces cerevisae	9763	30-300	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.

Antimicrobic Vial A

Antimicrobic Vial A contains 25 mg sterile desiccated chlortetracycline (Aureomycin®) per 10 ml vial.

Precautions

1. For Laboratory Use.
2. **Antimicrobic Vial A**

   HARMFUL. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Teeth, Bones.

   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.

Store Antimicrobic Vial A 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

Cooke Rose Bengal Agar
Antimicrobic Vial A

Materials Required but not Provided

Glassware
Autoclave
Sterile Petri dishes
Incubator
Waterbath (optional)

Method of Preparation

Antimicrobic Vial A

1. Aseptically add 10 ml sterile distilled or deionized water to Antimicrobic Vial A.
2. Agitate gently to dissolve completely.
3. The resulting concentration of the rehydrated solution is 2.5 mg chlortetrycycline per ml.

Cooke Rose Bengal Agar

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45°C.
5. OPTIONAL: To increase selectivity, aseptically add 14 ml of rehydrated Antimicrobic Vial A to achieve a final concentration of 35 µg of chlortetracycline per ml of medium or an appropriate amount of another antibiotic.

Specimen Collection and Preparation

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

Test Procedure

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

Results

Refer to appropriate references and procedures for results.

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. **Cooke.** 1922. J. Bact. **7:**339.
2. **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.
3. **Waksman.** 1954. Antibiotics and Chemotherapy **4:**657.
4. **Taplin, Azias, Rebell, and Blank.** 1969. Arch. Dermatol. **99**:203.

Packaging

Cooke Rose Bengal Agar	500 g	0703-17
Antimicrobic Vial A	6 x 10 ml	3333-60

Bacto® Cooked Meat Medium

Intended Use

Bacto Cooked Meat Medium is used for cultivating anaerobic microorganisms and for maintaining stock cultures.

Also Known As

Cooked Meat Medium (CMM) is also called Chopped Meat Medium.

Summary and Explanation

In 1890, Theobald Smith[1] made use of fresh unheated animal tissue for cultivating anaerobic organisms. Tarozzi[2] confirmed Smith's[1] findings and discovered the meat-broth could be heated to 104-105°C for 15 minutes without destroying medium nutrients. A steam sterilized emulsion of brain tissue in water was employed by von Hibler[3,4] for cultivating anaerobic microorganisms. Von Hibler[3,4] found organisms in cooked brain broth were less susceptible to harmful effects of toxic metabolic products than in

carbohydrate serum media. Robertson[5] substituted beef heart for brain tissue and found successful results. Cooked Meat Medium is prepared according to the formulation of Robertson.[5]

The capacity of Cooked Meat Medium to detoxify metabolic products of microorganisms makes it an excellent maintenance and growth medium. A study of various formulations used to grow and maintain clinical isolates of anaerobic bacteria found Chopped Meat Broth superior.[6]

Cooked Meat Medium's ability to initiate growth in a small inoculum makes it valuable for the primary culture of clinical specimens. Cooked Meat Medium can be supplemented with vitamin K_1 (1% alcohol solution) and hemin (1% solution) for clinical isolates.[7] This modification is used as a general enrichment for anaerobes, and as a backup for anaerobic jar or chamber failure.[7]

Chopped Meat Carbohydrate Medium and Chopped Meat Glucose Medium is used for cultivation and maintenance of anaerobic bacteria.[7,8,9] Cooked Meat Medium is recommended in the Bacteriological Analytical Manual[10] for use in the examination of *Clostridium botulinum* from food and in the Compendium of Methods for the Microbiological Examination of Foods.[11]

Principles of the Procedure

Beef Heart and Proteose Peptone provide the nitrogen, vitamins and amino acids in Cooked Meat Medium. Sodium Chloride maintains the osmotic balance of the medium. The low concentration of Dextrose is sufficient as the energy source, but not high enough to accumulate toxic metabolites. This formulation provides an effective maintenance medium.

Solid meat particles provide favorable growth conditions for anaerobes due to the reducing action of -SH (sulfhydryl) groups of muscle protein.[2,3,4] Sulfhydryl groups are more accessible in denatured proteins, therefore the use of cooked meat particles is preferred.[9]

Formula

Cooked Meat Medium

Formula Per Liter

Beef Heart	454 g
Bacto Proteose Peptone	20 g
Bacto Dextrose	2 g
Sodium Chloride	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Cooked Meat Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Distilled or deionized water
Sterile tubes with closures

User Quality Control

Identity Specifications

Dehydrated Appearance:	Brown pellets.
Prepared Medium:	Medium amber, clear supernatant over insoluble pellets.
Reaction of 12.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Cooked Meat Medium per label directions. Inoculate and incubate medium at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides vulgatus	8482	100-1,000	good
Clostridium novyi	7659	100-1,000	good
Clostridium perfringens	12924	100-1,000	good
Clostridium sporogenes	11437	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good

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

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

Uninoculated tube

Clostridium sporogenes ATCC® 11437

Method of Preparation

1. Suspend 12.5 grams in 100 ml distilled or deionized water (1.25 g per 10 ml).
2. Let stand until all particles are thoroughly wetted and form an even suspension.
3. Autoclave at 121°C for 15 minutes. Reduce pressure slowly.
4. Cool without agitation.
5. If not used within 24 hours, reheat (100°C) prior to use to drive off dissolved oxygen.

Specimen Collection and Preparation

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

Test Procedure

1. Inoculate specimen well into the meat particles (bottom of the tube). Tissue specimens should be ground prior to inoculation.
2. Growth is indicated by turbidity and/or the presence of gas bubbles.
3. For a complete discussion on the isolation and identification of aerobic and anaerobic bacteria, refer to appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for results.

Limitations

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. **Smith, T.** 1890. Centr. Bakteriol. **7**:509.
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4. **von Hibler, E.** 1908. Untersuchungen uber die pathogenen Anaerobier, Jena: Verlag Fischer.
5. **Robertson, M.** 1916. Notes upon certain anaerobes isolated from wounds. J. Pathol. Bacteriol. **20**:327.
6. **Claros, M. C., D. M. Citron, and E. J. C. Goldstein.** 1995. Survival of anaerobic bacteria in various thioglycollate and chopped meat broth formulations. J. Clin. Microbiol. **33**:2505-2507.
7. **Isenberg, H. E. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
8. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 224-226. CRC Press, Boca Raton, FL.
9. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 240-246. Williams & Wilkins, Baltimore, MD.
10. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
11. **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

Cooked Meat Medium	100 g	0267-15
	500 g	0267-17
	10 kg	0267-15r

Bacto® Corn Meal Agar

Intended Use

Bacto Corn Meal Agar is used for stimulating the production of chlamydospores by most strains of *Candida albicans* and for cultivating phytopathological fungi.

Summary and Explanation

Numerous culture media formulations have been described for the detection, isolation, and identification of *Candida albicans*, the etiological agent in candidiasis. The various media were designed to bring out morphological or physiological characteristics in this organism which would differentiate it from other members of the genus as well as from other genera.

One of the most important differential characteristics of *C. albicans* in its ability to form chlamydospores on certain media. This property is perhaps the best criterion for identification. Corn Meal Agar is valuable for morphologic differentiation of many yeast-like organisms. It suppresses vegetative growth of many fungi while stimulating sporulation.[1]

Corn Meal Agar has been used with varying degrees of success for showing chlamydospore formation in *C. albicans*. Chlamydospore production is the best diagnostic criterion for identification of the pathogenic yeast *C. albicans*.[2] Kelly and Funigeillo[3] reported that the addition of 1% Tween 80 enhanced chlamydospore formation by *C. albicans*. With this improvement, Corn Meal Agar may be the most accurate routine tool available for identification of *C. albicans*.[4]

Principles of the Procedure

Infusion from corn meal is a source of carbon, protein and nutrients. Bacto Agar is a solidifying agent.

Formula

Corn Meal Agar

Formula Per Liter

Corn Meal, Infusion from	50 g
Bacto Agar	15 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 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

Corn Meal Agar

Materials Required but not Provided

Glassware
Autoclave
Sterile Inoculating Needle
Cover Glass

Method of Preparation

1. Suspend 17 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. Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory according to recommended guidelines.[5]

Test Procedure[6]

1. Using a sterile inoculating needle, lightly touch the yeast colony, then make two streaks approximately 1.5 cm long each and 1.0 cm apart.
2. Flame the needle, and allow it to cool. Lightly make an S-shaped streak back and forth across the two streak lines.
3. Flame sterilize a cover glass. Allow it to cool, then place it over the streak marks.
4. Incubate at 22-26°C for 72 hours.

Results

1. Examine plates for the presence of chlamydospores.

User Quality Control

Identity Specifications

Dehydrated Medium	
Appearance:	Yellow, free-flowing, homogeneous.
Solution:	1.7% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent to opalescent, may have a slight, fine precipitate.
Reaction of 1.7% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Prepare Corn Meal Agar per label directions. Inoculate using the spread plate method. Prepare a heavy suspension of *C. albicans*, dip a sterile inoculating loop into the suspension, and cut a 2 cm "X" through the medium. Place a cover slip over the "X". Incubate at 20-25°C for 40-48 hours and up to four days, if required. Examine plates for chlamydospores which, when produced by some *Candida* species, appear as double walled spheres on cover slip plates.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	CHLAMYDOSPORES
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.

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

Limitations of the Procedure

1. Corn Meal Agar with the addition of 1% Tween 80 should not be the only medium used for identification of *C. albicans* since *C. stellatoidea* and *C. tropicalis* also produce chlamydospores on this medium.[7]
2. Repeated subculture of some *Candida* strains will result in the reduced ability to form chlamydospores.

References

1. **Baron, E. J., and S. M. Finegold.** 1990. Formulas and preparation of culture media and reagents, p. A-10. Bailey & Scott's Diagnostic Microbiology, 8th ed. The C. V. Mosby Company, St. Louis, MO.
2. **Duncan, J., and J. Floeder.** 1963. A comparison of media for the production of chlamydospores by Candida albicans. Am. J. Med. Tech. **29:**199-206.
3. **Kelly, J. P., and F. Funigiello.** 1959. Candida albicans: A study of media designed to promote chlamydospore production. J. Lab. & Clin. Med. **53**:807- 809.
4. **Gordon, M. A., and G. N. Little**.1963. Effective dehydrated media with surfactants for identification of Candida albicans. J. of Int. Soc. for Human and Animal Mycol. **2**:171-175.
5. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection and handling, p. 19- 32. *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.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
7. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 247-250. Williams & Wilkins, Baltimore, MD.

Packaging

Corn Meal Agar	500 g	0386-17

Bacto® Cystine Heart Agar

Intended Use

Bacto Cystine Heart Agar is used with Bacto Hemoglobin for cultivating *Francisella tularensis* and without enrichment for cultivating gram-negative cocci and other microorganisms.

Also Known As

Cystine Heart Agar with added hemoglobin is also referred to as Cystine Glucose Blood Agar.

Summary and Explanation

Francisella tularensis was first described in humans in 1907.[1] Several media formulations were employed to isolate this microorganism. Initial formulations contained egg or serum and were difficult to prepare. Edward Francis,[2] who dedicated his career to the study of this organism, reported that blood dextrose cystine agar was a satisfactory medium for cultivating this fastidious pathogen. Shaw[3] added 0.05% cystine and 1% dextrose to Heart Infusion Agar for the cultivation of *F. tularensis*.

While experimenting with Francis' blood dextrose cystine agar, Rhamy[4] added hemoglobin to Cystine Heart Agar to develop a satisfactory medium for growth of *F. tularensis*.

Cystine Heart Agar is the medium of choice for isolating *F. tularensis*.[1,5]

Principles of the Procedure

Infusions from Beef Heart, Proteose Peptone and L-Cystine provide nitrogen, vitamins and amino acids in Cystine Heart Agar. Dextrose is a carbon source. Sodium chloride maintains the osmotic balance and Bacto Agar is a solidifying agent.

Enrichment with 2% hemoglobin provides additional growth factors. Without enrichment, Cystine Heart Agar supports excellent growth of gram-negative cocci and other pathogenic microorganisms.[6] Rabbit blood and antimicrobial agents can be added to this medium.[5]

Formula

Cystine Heart Agar

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	10 g
Sodium Chloride	5 g
L-Cystine	1 g
Bacto Agar	15 g

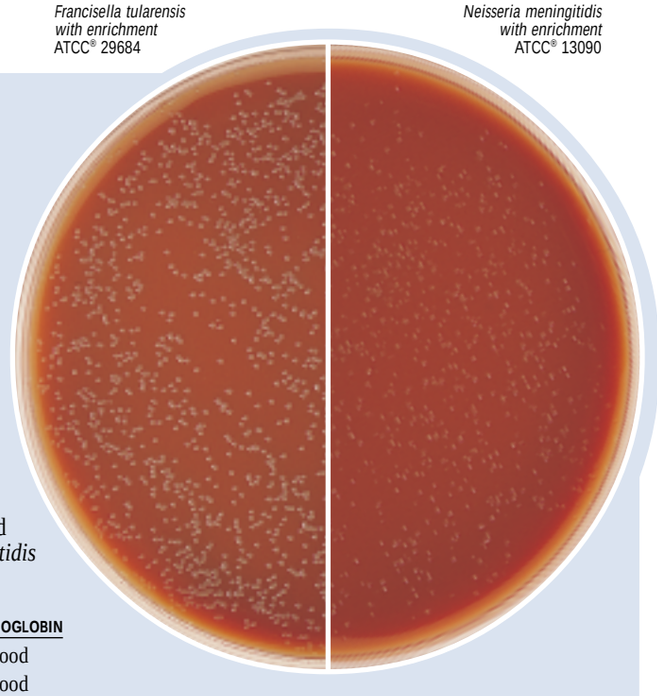
Final pH 6.8 ± 0.2 at 25%C

Precautions

1. For Laboratory Use.
2. *Francisella tularensis* is a Biosafety Level 2 pathogen that can be transmitted by aerosols or by penetration of unbroken skin.[5] Wearing of gowns, gloves and masks is advocated for laboratory staff handling suspected infectious material.
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.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in distilled or deionized water upon boiling; light to medium amber, very slightly to slightly opalescent, may have fine precipitate.
Prepared Medium:	Plain - Light to medium amber, slightly opalescent, may have fine precipitate.
	With Hemoglobin - Chocolate, opaque.
Reaction of 5.1% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Cystine Heart Agar per label directions. Incubate inoculated medium at 35 ± 2°C aerobically for 18-48 hours. *Neisseria meningitidis* should be incubated under 5- 10% CO_2.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o HEMOGLOBIN	GROWTH w/HEMOGLOBIN
Francisella tularensis	29684	100-1,000	fair	good
Neisseria meningitidis	13090*	100-1,000	good	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.

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

Cystine Heart Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Hemoglobin Solution 2% or Hemoglobin (optional)
Sterile Petri dishes or tubes

Method of Preparation

Enriched Medium:

1. Suspend 10.2 grams in 100 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-60°C.
4. Add 100 ml sterile 2% hemoglobin solution and mix well. Use:
   - Hemoglobin Solution 2%; or,
   - Prepare a 2% hemoglobin solution as follows: Place 2 grams of Hemoglobin in a dry flask. Add 100 ml of cold distilled or deionized water while agitating vigorously. Continue intermittent agitation for 10-15 minutes until solution is complete. Autoclave at 121°C for 15 minutes. Cool to 50-60°C
5. Dispense into sterile Petri dishes or tubes.

Unenriched Medium:

1. Suspend 51 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs. Transport immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

1. Inoculate and streak specimens as soon as possible. For a complete discussion on the inoculation and identification of *Francisella*, consult appropriate references.
2. Overgrowth by contaminating organisms can be reduced by incorporating 100- 500 units penicillin per ml into the medium.[6]

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. **Stewart, S. J**. 1995. *In* P. R. Murray., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Francis, E.** 1928. Symptoms, diagnosis and pathology of tularemia. J. Am. Med. Assoc. **91**:1155-1160.
3. **Shaw, F. W.** 1930. Culture medium for *Bacterium tularense*. Zentr. Bakt. I. Abt. Orig. **118**:216-217.
4. **Rhamy, B. W.** 1933. A new and simplified medium for *Pasteurella tularensis* and other delicate organisms. Am. J. Clin. Pathol. **3**:121-124.
5. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
6. **Stewart, S. J.** 1995. *Francisella*, p. 545-548. *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.

Packaging

Cystine Heart Agar	500 g	0047-17
Hemoglobin	100 g	0136-15
	500 g	0136-17
	2 kg	0136-07
	10 kg	0136-08
Hemoglobin Solution 2%	6 x 100 ml	3248-73

Bacto® Cystine Tryptic Agar

Intended Use

Bacto Cystine Tryptic Agar is used with added carbohydrates in differentiating microorganisms based on fermentation reactions and motility.

Also Known As

Cystine Tryptic Agar is abbreviated as CTA, and referred to as CT Medium.

Summary and Explanation

Cystine Tryptic Agar is a semi-solid basal medium prepared according to the formula of Vera.[1] Many tests used to differentiate among members of the *Enterobacteriaceae* determine the organism's ability to utilize a carbohydrate with the production of acid metabolic end products.[2] CTA is free of fermentable carbohydrates, and the carbohydrate content can be adjusted for specific reactions. The carbohydrate concentration used most frequently in fermentation reactions is 0.5 or 1%.

Some researchers prefer 1% to insure against reversion of the reaction due to depletion of the carbohydrate by the microorganism.

The low agar content of Cystine Tryptic Agar provides a suitable environment for motility studies. Motility determination aids in the identification of bacteria. CTA can also be used as a maintenance medium for stock cultures.[3,4] This formula will support the growth of fastidious organisms, e.g., *Streptococcus pneumonia* and *Corynebacterium* species.[4]

Principles of the Procedure

Tryptose provides the nitrogen, vitamins and amino acids in Cystine Tryptic Agar. L-Cystine and Sodium Sulfite are added to this formula to stimulate growth. Sodium Chloride maintains the osmotic balance of the medium. Phenol Red is the pH indicator. Bacto Agar maintains an Eh potential which facilitates anaerobic growth, and aids in dispersion of reducing substances and CO_2 formed in the environment.[5] The agar is also used for the determination of motility.

Formula

Cystine Tryptic Agar

Formula Per Liter

Bacto Tryptose	20 g
L-Cystine	0.5 g
Sodium Chloride	5 g
Sodium Sulfite	0.5 g
Bacto Agar	2.5 g
Bacto Phenol Red	0.017 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

Cystine Tryptic Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (50-55°C) (optional)
Sterile 5-10% carbohydrate solution
Sterile tubes

Method of Preparation

1. Suspend 28.5 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. To prepare fermentation medium, use one of the following methods:
   A. Add 5-10 grams carbohydrate before sterilization.
   or B. Dissolve 28.5 grams medium in 900 ml water, sterilize and aseptically add 100 ml sterile of carbohydrate solution.

User Quality Control

Identity Specifications

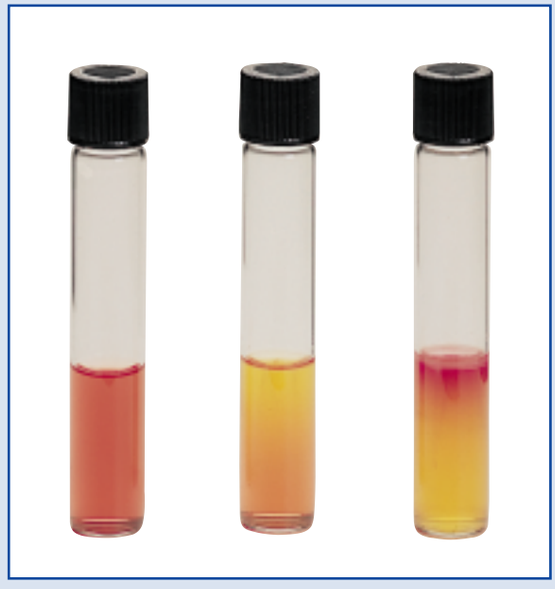
Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	2.85% solution, soluble in distilled or deionized water upon boiling. Red, very slightly opalescent without significant precipitate.
Prepared Medium:	Red, very slightly opalescent without precipitate.
Reaction of 2.85% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Cystine Tryptic Agar per label directions with and without 0.5% dextrose. Inoculate tubes by straight stab and incubate at 35°C for 18-48 hours.

ORGANISM	ATCC®	MOTILITY	ACID PRODUCTION w/ DEXTROSE
Corynebacterium diphtheriae subsp. *mitis*	8024	–	+
Escherichia coli	25922*	+	+
Neisseria gonorrhoeae (CDC 98)	43070*	–	+

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 | *Corynebacterium diptheriae* ATCC® 8024 with dextrose | *Escherichia coli* ATCC® 25922 with dextrose

Specimen Collection and Preparation

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

Test Procedure

For a complete discussion on motility and carbohydrate fermentation studies refer to procedures described in appropriate references.[2,6,7]

Results

1. Fermentation of the test carbohydrate is observed when acid is formed and the medium turns from red to yellow.
2. Motility of an organism is evident as a haze of growth extending into the agar from the stab line.[2]

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. CTA requires a heavy inoculum.[5]
3. Prolonged incubation may lead to changes in pH indicator or abnormal lactose/sucrose reactions with Neisseria pathogens.[8,9]
4. *Neisseria* species usually produce acid only in the area of stabs (upper third). If there is a strong acid (yellow color) throughout the medium, a contaminating organism may be present. If in doubt about a tube containing a *Neisseria* species, a Gram stain and oxidase test should be performed on the growth.[5]

References

1. **Vera, H. D.** 1948. A simple medium for identification and maintenance of the gonococcus and other bacteria. J. Bacteriol. **55**:531.
2. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
3. **Myers, R. M., and G. Koshy.** 1961. Beta-hemolytic streptococci in survey throat cultures in an Indian population. Am. J. Public Health **51**:1872.
4. **Alford, J. A., G. E. Wiese, and J. J. Gunter.** 1955. Heat resistance in *Corynebacterium* and the relationship of the genus to *Microbacterium*. J. Bacteriol. **69**:516.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 254-259, 802-804. Williams & Wilkins, Baltimore, MD.
6. **Isenberg, H. D. (ed.).** 1995. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Carbohydrate fermentation plate medium for confirmation of *Neisseria* species. J. Clin. Microbiol. **1**:294.
9. **Applebaum, P. C., and R. B. Lawrence.** 1979. Comparison of three methods for identification of pathogenic *Neisseria* species. J. Clin. Microbiol. **9**:598.

Packaging

Cystine Tryptic Agar	500 g	0523-17

Bacto® Czapek-Dox Broth Bacto Czapek Solution Agar

Intended Use

Bacto Czapek-Dox Broth and Czapek Solution Agar are used for cultivating fungi and bacteria capable of using inorganic nitrogen.

Summary and Explanation

Czapek-Dox Broth and Czapek Solution Agar are a modification of the Czapek[1] and Dox[2] formula prepared according to Thom and Raper.[3] The media are prepared with only inorganic sources of nitrogen and chemically defined compounds sources of carbon. Czapek-Dox media are useful in a variety of microbiological procedures, including soil microbiology and fungi and mildew resistance tests. Thom and Raper[3] reported Czapek-Dox Broth and Czapek Solution Agar will produce moderately vigorous growth of most saprophytic aspergilli and yield characteristic mycelia and conidia.

Czapek Solution Agar is recommended in Standard Methods for the Examination of Water and Wastewater[5] for the isolation of *Aspergillus*, *Penicillium* and related fungi.

Principles of the Procedure

Saccharose is the sole carbon source, and Sodium Nitrate is the sole nitrogen source in Czapek-Dox Broth and Czapek Solution Agar. Dipotassium Phosphate is the buffering agent, and Potassium Chloride contains essential ions. Magnesium Sulfate and Ferrous Sulfate sources of cations. Bacto Agar is the solidifying agent in Czapek Solution Agar.

Formula

Czapek-Dox Broth

Formula Per Liter	
Bacto Saccharose	30 g
Sodium Nitrate	3 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Potassium Chloride	0.5 g
Ferrous Sulfate	0.01 g

Final pH 7.3 ± 0.2 at 25°C

Czapek Solution Agar

Formula Per Liter	
Bacto Saccharose	30 g
Sodium Nitrate	2 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Potassium Chloride	0.5 g
Ferrous Sulfate	0.01 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

Czapek-Dox Broth

1. For Laboratory Use.

Czapek Solution Agar

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

Storage

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

Expiration Date

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

User Quality Control

Identity Specifications

Czapek-Dox Broth

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water. Solution is colorless, clear to very slightly opalescent and may have a slight precipitate.
Prepared Medium:	Colorless, clear to very slightly opalescent, may have slight precipitate.
Reaction of 3.5% Solution at 25°C:	pH 7.3 ± 0.2

Czapek Solution Agar

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	4.9% solution, soluble in distilled or deionized water on boiling; light amber, opalescent with a uniform flocculent precipitate.
Prepared Medium:	Light amber, slightly opalescent; may have slight precipitate.
Reaction of 4.9% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Czapek-Dox Broth

Prepare Czapek-Dox Broth per label directions. Inoculate tubes with the test organisms. Incubate inoculated medium at 30 ± 2°C for 48-72 hours.

Czapek Solution Agar

Prepare Czapek Solution Agar per label directions. Inoculate prepared medium with the test organisms. Incubate at 30 ± 2°C for 18-48 hours, or up to 72 hours if necessary.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	9642	100-1,000	good
Candida albicans	10231	100-1,000	good

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

Procedure

Materials Provided

Czapek-Dox Broth
Czapek Solution Agar

Materials Required but not Provided

Glassware
Autoclave
Incubator
Waterbath (optional)

Method of Preparation

Czapek-Dox Broth

1. Dissolve 35 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Dispense as desired.

Czapek Solution Agar

1. Suspend 49 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense as desired.

Specimen Collection and Preparation

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

Test Procedure

Refer to appropriate references for specific procedures for the cultivation of fungi and bacteria capable of utilizing inorganic nitrogen.

Results

Refer to appropriate references and procedures for results.

References

1. **Czapek, F.** 1902-1903. Untersuchungen uber die stickstoffgewinnung und EiweiBbildung der Pflanze. Beitr. Chem. Physiol. Pathol. **1**:540.
2. **Dox, A. W.** 1910. The intracellular enzymes of *Penicillium* and *Aspergillus* with special references to those of *P. camenberti*. U.S. Dept. Agr. Bur. Anim. Ind. Bull. **120**:70.
3. **Thom, C., and K. B. Raper.** 1945. Manual of the aspergilli, vol. 39.
4. **Thom, C., and M. B. Church.** 1926. The aspergilli. Williams and Wilkins Co., Baltimore, MD.
5. **Eaton, A. D., L. S. Cleseri, 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

Czapek-Dox Broth	500 g	0338-17
Czapek Solution Agar	500 g	0339-17