Bacto® m T7 Agar

Intended Use

Bacto mT7 Agar is used for recovering injured coliforms from treated water by membrane filtration.

Summary and Explanation

Selective media used with the membrane filter method do not adequately recover injured coliforms.[1,2,3,4] McFeters et al. studied the influences of diluents, media and procedures in recovering injured coliform bacteria and found improved recovery using Tergitol 7 Agar.[5] LeChevallier et al. modified Tergitol 7 Agar and developed a new medium, m T7 Agar, for improved recovery of injured coliforms from drinking water.[6] In a later study, LeChevallier et al.[7] evaluated mT7 Agar as a fecal coliform medium and found optimum recovery using preincubation at 37°C for 8 hours followed by incubation at 44.5°C for 12 hours.[7] The authors found that incorporation of 0.1 µg of penicillin G per ml, aseptically added to the medium after autoclaving, prevented growth of gram-positive cocci that may break through. Later, they found that 1.0 µg/ml of penicillin G provided far better inhibition of gram-positive organisms without interfering with the recovery of coliforms. LeChevallier and McFeters reported the work of five collaborating laboratories testing coliform recovery from contaminated surface water and sewage samples.[8] They found m T7 Agar to be more effective than m Endo Agar in recovering coliforms.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Yellow-green to blue-green, free-flowing, homogeneous, may have a slightly moist appearance and/or tendency to form soft lumps.
Solution:	4.86% solution, soluble in distilled or deionized water upon boiling. Reddish purple, slightly opalescent without significant precipitate.
Prepared Medium:	Reddish purple, slightly opalescent without significant precipitate.
Reaction of 4.86% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare mT7 Agar per label directions. Inoculate with test organisms diluted in 10 ml of water. Incubate at 35 ± 2°C for 8 hours and then at 44.5°C for an additional 12 hours.

ORGANISM	ATCC®	INOCULUM CFU (approx.)	GROWTH	COLONY COLOR
Escherichia coli	25922*	100	good	yellow
Escherichia coli	13762	100	good	yellow
Enterococcus faecalis	19433	100	poor to fair	–
Pseudomonas aeruginosa	27853*	100	poor to fair	–

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.

Standard Methods procedures to recover injured total coliform bacteria from treated water specify m T7 Agar.[9] Stressed organisms can be present in treated drinking water and wastewater, saline waters and relatively clean surface waters.[9]

Principles of the Procedure

The ingredients of m T7 Agar support growth of injured coliforms. Proteose Peptone No. 3 provides nitrogen and amino acids. Yeast Extract is a vitamin source and Lactose provides carbon. Tergitol 7 and Polyoxyethylene Ether W-1 are selective agents at optimal concentrations that will not affect recovery of injured coliforms. Brom Cresol Purple and Brom Thymol Blue are indicators of lactose fermentation. The combination of dyes provides a good differential reaction as well as additional inhibition to noncoliform bacteria. Bacto Agar is a solidifying agent.

Penicillin G (1.0 µg/ml), aseptically added to the medium after autoclaving, prevents growth of gram-positive cocci without interfering with recovery of coliforms.[8]

Formula

m T7 Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	20 g
Tergitol 7	0.4 ml
Polyoxyethylene Ether W-1	5 g
Bacto Brom Thymol Blue	0.1 g
Bacto Brom Cresol Purple	0.1 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 prepared plates containing penicillin G at 2-8°C and use within 1 week after preparation.[6]

Expiration Date

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

Procedure

Materials Provided

m T7 Agar

Materials Required But Not Provided

Glassware
Autoclave
Distilled or deionized water

Membrane filter equipment
Sterile 47 mm 0.45 µm gridded membrane filters
Sterile Petri dishes 50 x 9 mm
Pipettes
Stereoscopic microscope
Dilution bottles
Incubator or waterbath (37°C and 45°C)
Penicillin G (1.0 µg/ml)

Method of Preparation

1. Suspend 48.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. To prepare a more selective medium, aseptically add 1.0 µg penicillin G per ml to the sterile medium cooled to 45°C.
5. Dispense 4-5 ml amounts into 50 x 9 mm Petri dishes.

Note: Stock solutions of 0.1 mg/ml of penicillin G (sodium salt) can be filter sterilized, frozen in aliquots, and stored for up to 6 months. (One international or USP penicillin unit is equivalent to 0.6 µg of benzylpenicillin sodium).

Specimen Collection and Preparation

Water samples should be collected as described in Standard Methods for the Examination of Water and Wastewater.[9]

Test Procedure

For a complete discussion of stressed organisms in water testing, refer to the membrane filter procedure for the coliform group as described in Standard Methods for the Examination of Water and Wastewater.[9]

Incubate inoculated plates at 37°C for 8 hours and then at 44.5°C for an additional 12 hours. This procedure has been found to produce consistently higher fecal coliform counts with mT 7 Agar.[7]

Results

After incubation, count all yellow, smooth, convex colonies as coliforms with the aid of a stereoscopic microscope.

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 procedure for enumerating fecal coliforms with m T7 Agar requires two incubation temperatures.
3. The addition of penicillin G is required for better inhibition of gram-positive bacteria.
4. m T7 Agar may recover other coliforms in addition to *E. coli*. Some drinking water samples contain so many non-coliform bacteria that confluent growth may occur. Care must be taken to distinguish yellow colonies from background growth.[9]

References

1. **Maxcy, R. B.** 1970. Non-lethal injury and limitations of recovery of coliform organisms on selective media. J. Milk Food Technol. **33**:445-448.
2. **Scheusner, D. L., F. F. Busta, and M. L. Speck.** 1971. Inhibition of injured *Escherichia coli* by several selective agents. Appl. Microbiol. **21**:46-49.
3. **Grabow, W. O. K., and M. du Preez.** 1979. Comparison of mEndo LES, MacConkey and Teepol media for membrane filtration counting of total coliform bacteria in water. Appl. Environ. Microbiol. **38**:351-358.
4. **Hoadley, A. W., and C. M. Cheng.** 1974. Recovery of indicator bacteria on selective media. J. Appl. Bacteriol. **37**:45-57.
5. **McFeters, G. A. , S. C. Cameron, and M. W. LeChevallier.** 1982. Influence of diluents, media and membrane filters on detection of injured waterborne coliform bacteria. Appl. Environ. Microbiol. **43**:97-103.
6. **LeChevallier, M. W., S. C. Cameron, and G. A. McFeters.** 1983. New medium for improved recovery of coliform bacteria from drinking water. Appl. Environ. Microbiol. **45**:484-492.
7. **LeChevallier, M. W., P. E. Jajanoski, A. K. Camper, and G. A. McFeters.** 1984. Evaluation of m-T7 agar as a fecal coliform bacteria from drinking water. Appl. Environ. Microbiol. **48**:371-375.
8. **LeChevallier, M. W., and G. A. McFeters.** 1985. Enumerating injured coliforms in drinking water. Research and Technology. J. AWWA. **77**:81-87.
9. **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

mT7 Agar	100 g	0018-15

Bacto® TAT Broth Base
Bacto TAT Broth

Intended Use

Bacto TAT Broth Base with added Tween® 20 and Bacto TAT Broth are used for cultivating microorganisms from highly viscous or gelatinous materials.

Also Known As

TAT (Tryptone-Azolectin-Tween) Broth Base is also referred to as Fluid Casein Digest-Soy Lecithin Polysorbate 20 Medium.

Summary and Explanation

TAT Broth Base with the addition of Tween® 20 is recommended for sterility testing of viscous materials, such as salves or ointments. It is especially adapted to the sterility testing of cosmetics. Cosmetics and pharmaceutical products are subject to contamination during manufacturing and use by consumers.[1] Preservatives are used in aqueous products to make them self-sterilizing for vegetative bacteria, yeasts and molds.[1]

TAT Broth Base is an enrichment medium developed to isolate and cultivate microorganisms. TAT Broth Base conforms to the formula specified by US Pharmacopeia for use in Microbial Limit Tests.[2]

Principles of the Procedure

Tryptone provides the nitrogen, vitamins, amino acids and carbon in TAT Broth Base. Azolectin and Tween® 20 neutralize preservatives in the cosmetics or pharmaceutical products, allowing bacteria to grow.

Formula

TAT Broth Base

Formula Per Liter

Bacto Tryptone	20 g
Azolectin	5 g

Final pH 7.2 ± 0.2 at 25°C

TAT Broth

Formula Per Liter

Bacto Tryptone	20 g
Azolectin	5 g
Tween® 20	40 ml

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.

Store the prepared medium at 15-30°C.

User Quality Control

Identity Specifications

TAT Broth Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.5% solution with 4% Tween® 20; solution is light amber, clear to very slightly opalescent with a very slight precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent.
Reaction of 2.5% Solution w/ 4% Tween® 20 at 25°C:	pH 7.2 ± 0.2

Cultural Response

TAT Broth

Prepare TAT Broth Base per label directions or use prepared TAT Broth. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Pseudomonas aeruginosa	27853*	100-1,000	good
Salmonella typhi	6539	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 Disk 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

TAT Broth Base (dehydrated)
TAT Broth (prepared)

Materials Required But Not Provided

Tween® 20 (for dehydrated TAT Broth Base)
Glassware
Autoclave
Waterbath (50-60°C)
Sterile test tubes

Method of Preparation

TAT Broth Base (dehydrated)

1. Suspend 25 grams in 960 ml distilled or deionized water.
2. Add 40 ml Tween® 20.
3. Heat to 50-60°C.
4. Let stand 15-30 minutes with occasional agitation to dissolve completely.
5. Autoclave at 121°C for 15 minutes.
6. Dispense as desired.

TAT Broth (prepared)

1. In an area adjacent to the clean room, remove bottles from their boxes.
2. Follow careful aseptic technique when uncapping bottles for testing.

Specimen Collection and Preparation

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

Test Procedure

1. Add one gram or one ml of an undiluted sample to 40 ml of complete medium and agitate to obtain an even suspension.
2. Incubate tubes at 35 ± 2°C for 18-48 hours.

For a complete discussion on sterility testing refer to appropriate procedures in USP.[2]

Results

Tubes or bottles exhibiting growth should be subcultured for identification.

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. **Orth, D. S.** 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, N.Y.
2. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. Microbial limits tests, p. 1681-1686. The United States Pharmacopeial Convention Inc., Rockville, MD.

Packaging

TAT Broth Base	500 g	0984-17
TAT Broth	10 x 90 ml	9072-73

Bacto® TB Hydrolysis Reagent

Intended Use

Bacto TB Hydrolysis Reagent is used for differentiating mycobacteria on their ability to hydrolyze Polysorbate 80. TB Hydrolysis Reagent is also used for differentiating *Moraxella catarrhalis* from *Neisseria* spp.

Also Known As

"Tween hydrolysis" is a common term for the TB Hydrolysis Reagent test.

Summary and Explanation

Wayne, Doubek and Russell[1] differentiated various species and subgroups of acid-fast bacilli using the Tween 80 hydrolysis test. Kubica and Dye[2] used the test to differentiate clinically significant from clinically insignificant mycobacteria. In 1970, Runyan, Kubica, Morse, Smith and Wayne[3] defined a positive reaction as one that occurs in less than five days. A doubtful reaction was defined as one that occurs in five to ten days. A negative reaction was defined as one occurring after ten days.

In 1973, Kubica[4] recognized the greater reliability of a 10-day Tween hydrolysis test for separation of clinically insignificant from clinically significant members of both the scotochromagenic and non-photochromogenic mycobacteria. These observations were later confirmed by Wayne *et al*.[5] citing that a 10-day reading was regarded as a better end point for the Tween hydrolysis test.

In 1990, Weiner and Penha[5] described the differentiation of *Moraxella catarrhalis* from other *Moraxella* and *Neisseria* spp. using TB Hydrolysis Reagent.

Principles of the Procedure

Polysorbate 80 binds to the neutral red indicator, causing the solution to be amber colored. If the mycobacterial lipase splits the Polysorbate 80, it can no longer complex with the neutral red indicator which then exhibits its normal red color at pH 7. The intensity of the red depends upon how much Polysorbate 80 is split.

Some mycobacteria possess a lipase capable of splitting Polysorbate 80 into oleic acid and polyoxyethylated sorbitol, modifying the solution from yellow to pink. The differential criterion of this test is based on the relative time necessary for a particular species or subgroup to hydrolyze the compound. Most *M. kansasii*[2] strains and clinically insignificant species are positive in five days[4,5] or less, while clinically significant species may be negative even after three weeks. *Mycobacterium tuberculosis* generally yields a positive reaction in 10-20 days.

Moraxella catarrhalis hydrolyzes Tween 80 after 24 hours of incubation, producing a clear change in color from amber to pink-red. Other *Moraxella* and *Neisseria* spp. remain negative after an additional 24 hours of incubation.[6]

User Quality Control

Identity Specifications

Reagent Appearance: Reddish-amber solution

Cultural Response

The TB Hydrolysis Test is performed on cultures which are inoculated and incubated at 35 ± 2°C for 5-10 days for *Mycobacterium* sp. and 18-48 hours for *Moraxella* and *Neisseria* spp.

ORGANISM	ATCC®	TIME TO COLOR CHANGE	REACTION
Mycobacterium gordonae	14470	5 days	positive
Mycobacterium kansasii	12478	5 days	positive
Mycobacterium scrofulaceum	19981	10 days	negative
Moraxella (Branhamella) catarrhalis	25238	24 hours	positive
Neisseria sicca	9913	48 hours	negative

Positive - any change in reagent color from the original color

Negative - no change in color

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

Formula

TB Hydrolysis Reagent is a sterile, phosphate-buffered solution of Tween 80 and neutral red.

Precautions

1. CAUTION: Laboratory acquired infection is always a distinct possibility when handling and processing specimens containing *Mycobacterium tuberculosis*. Laboratory procedures with specimens containing *M. tuberculosis* should be performed in a properly equipped laboratory (*i.e.*, under a Class 1 negative pressure or Class 2 laminar flow biological safety cabinet) and by personnel thoroughly familiar with proper techniques. For detailed information, consult the appropriate references.[7,8,9]
2. For In Vitro Diagnostic Use.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store TB Hydrolysis Reagent 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

TB Hydrolysis Reagent

Materials Required But Not Provided

13 x 75 mm screw cap test tubes
Bacteriological loop
Incubator (35°C)
White paper or porcelain

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory according to recommended guidelines.[7]

2. Process each specimen, using procedures appropriate for that sample.[7]
3. Test actively metabolizing 3-4 week old pure cultures of *Mycobacterium*, or an 18-24 hour isolate of *Moraxella* spp. Carefully exclude underlying culture medium.

Test Procedure

1. Prepare and sterilize 13 x 75 mm screw cap test tubes containing 1 ml distilled or deionized water. Cool to room temperature.
2. Add two drops TB Hydrolysis Reagent, taking care not to touch the glass dropper, which could contaminate the reagent and cause aberrant test results.
3. Transfer one loopful of test culture to the tube. Thoroughly emulsify the culture in the reagent.
4. When testing *Mycobacterium* spp.:
   a. use a known positive (*M. kansasii* ATCC® 12478) and negative (uninoculated tube and *M. scrofulaceum*) control in parallel with the test culture to ascertain the validity of test results.
   b. incubate at 35 ± 2°C in the dark with caps tight for 5-10 days.
   c. read tubes at 5 and 10 days for any change in color in a strong light against a white background.
5. When testing *Moraxella* and *Neisseria* spp.:
   a. use *Moraxella catarrhalis* ATCC® 25238 for a positive control, and *Neisseria sicca* ATCC® 9913 as a negative control.
   b. incubate at 35 ± 2°C in the dark with caps tight for 18-48 hours.
   c. read tubes at 24 and 48 hours.
6. Do not shake the tubes. Examine the liquid, not the sedimented cells. Compare the color of the liquid with the control tube color.
7. Record results.
8. Upon completion of the test, follow proper established laboratory procedures in disposing of infectious materials.

Results

For *Mycobacterium* spp. - A positive reaction is indicated by a color change of the solution from amber to pink or red in 5 days or less. A doubtful reaction is a color change in 5 to 10 days. A negative reaction is no color change after 10 days.

For *Moraxella* and *Neisseria* spp. - A positive reaction is a color change of the solution from amber to red or pink after 24 hours of incubation. A negative reaction is no color change after 48 hours of incubation.

References

1. **Wayne, L. G., J. R. Doubek, and R. L. Russell.** 1964. Classification and identification of mycobacteria. 1. Tests employing Tween 80 as substrate, Am. Rev. Respir. Dis. **90:**588-597.
2. **Kubica, G. P., and W. E. Dye.** 1967. Laboratory methods for clinical and public health, mycobacteriology, p. 44. National Communicable Disease Center, Atlanta, Georgia.
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4. **Kubica, G. P.** 1973. Differential identification of mycobacteria. Am. Rev. Respir. Dis. **107:**9-21.
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6. **Weiner, M., and P. D. Penha.** 1990. Evaluation of Bacto TB Hydrolysis Reagent (Tween 80) for the identification of *Branhamella catarrhalis*. J. Clin. Microbiol. **28:**126-127.
7. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 2. American Society for Microbiology, Washington, D.C.
8. **Strain, B. A., and D. M. Grochel.** 1995 Laboratory safety and infectious waste management, p. 75-85. *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.
9. **Kent, P. T., and G. P. Kubica.** 1985. Public Health Mycobacteriology, p. 5-20. Centers for Disease Control, Atlanta, Georgia.

Packaging

TB Hydrolysis Reagent	5 ml	3192-56*

*Store at 2-8°C

Bacto® TCBS Agar

Intended Use

Bacto TCBS Agar is used for isolating and cultivating *Vibrio cholerae* and other enteropathogenic vibrios.

Also Known As

TCBS Agar is an abbreviation for Thiosulfate-Citrate-Bile-Sucrose Agar. TCBS is also called Vibrio Selective Agar.

Summary and Explanation

TCBS Agar, prepared according to the formula of Kobayashi *et al.*[1], is a modification of the selective medium from Nakanishi.[2] All *Vibrio* spp. that are pathogenic to humans, except *V. hollisae*, will grow on TCBS Agar. This medium is recommended for isolating *Vibrio* spp. from stool specimens[3] and is specified in Standard Methods as Thiosulfate-Citrate-Bile-Sucrose Agar for food testing.[4,5]

TCBS Agar is highly selective, meets the nutritional requirements of *Vibrio* spp., and allows vibrios to compete with intestinal flora. All members of the genus are able to grow in media containing increased salt concentrations and some species are halophilic.[6] Vibrios are natural inhabitants of sea water.[6] Human disease has been associated with ingestion of contaminated water and consumption of contaminated shellfish or seafood.

V. cholerae is the etiologic agent of a secretory diarrhea spread by the fecal-oral route.[3] Infections may be asymptomatic, mild, or severe.[3] If not treated, patients with severe cholera may die within 5 hours as a result of massive fluid and electrolyte loss.[3]

Seven cholera pandemics have been reported since 1817.[7] In 1993, the first reports of epidemic cholera due to a new serogroup,

non-01 cholerae, appeared.[8,9] This strain was designated *V. cholerae* 0139 and given the synonym Bengal.[3]

Principles of the Procedure

Yeast Extract and Proteose Peptone No. 3 provide the nitrogen, vitamins, and amino acids in TCBS Agar. Sodium Citrate, Sodium Thiosulfate and Oxgall are selective agents which provide an alkaline pH to inhibit gram-positive organisms and suppress coliforms. The pH of the medium is increased to enhance growth of *Vibrio cholerae* because this organism is sensitive to acid environments. Saccharose is a fermentable carbohydrate, and Sodium Chloride stimulates growth. Sodium Thiosulfate is a sulfur source and acts with Ferric Citrate as an indicator to detect hydrogen sulfide production. Brom Thymol Blue and Thymol Blue are pH indicators. Bacto Agar is a solidifying agent.

Formula

TCBS Agar

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Proteose Peptone No. 3	10 g
Sodium Citrate	10 g
Sodium Thiosulfate	10 g
Bacto Oxgall	8 g
Bacto Saccharose	20 g
Sodium Chloride	10 g
Ferric Citrate	1 g
Bacto Brom Thymol Blue	0.04 g
Thymol Blue	0.04 g
Bacto Agar	15 g

Final pH 8.6 ± 0.2 at 25°C

Precautions

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

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

Storage

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

Expiration Date

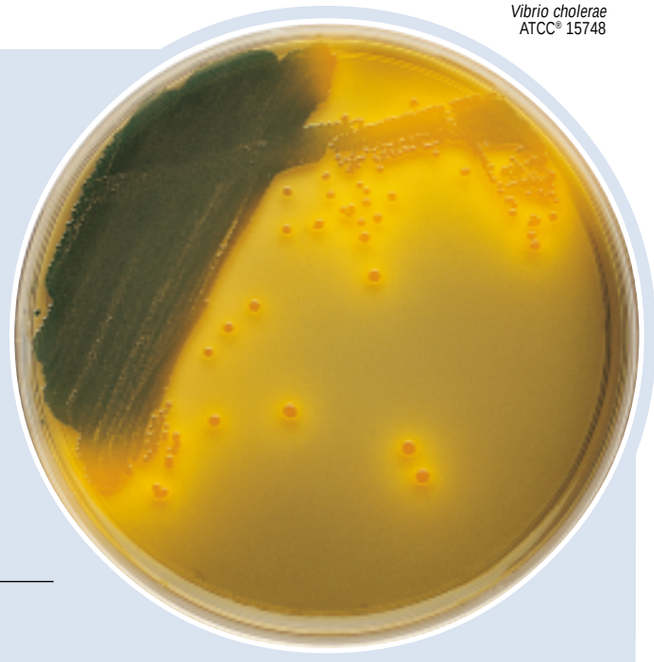
The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TCBS Agar

Vibrio cholerae
ATCC® 15748



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan with greenish cast, free-flowing, homogeneous.
Solution:	8.9% solution, soluble on boiling in distilled or deionized water. Solution is forest green and very slightly opalescent.
Prepared Medium:	Green, slightly opalescent.
Reaction of 8.9% Solution at 25°C:	pH 8.6 ± 0.2

Cultural Response

Prepare TCBS Agar per label directions. Inoculate the medium with 10 microliters (l) of a heavy suspension and incubate at 35°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM (HEAVY SUSPENSION)	GROWTH	COLONY COLOR
Escherichia coli	25922*	10 µl	inhibited	–
Vibrio cholerae El Tor	15748	10 µl	good	yellow
Vibrio parahaemolyticus		10 µl	good	blue green

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.

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 89 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. DO NOT AUTOCLAVE.
3. Cool to 45-50°C.
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. If any delay in culturing is anticipated, addition of the specimen to Cary-Blair transport medium is essential because *Vibrio* spp. are particularly susceptible to drying.[3] Inoculation into alkaline peptone water is acceptable if subculture will be within 6-8 hours.[10]

Test Procedure

For a complete discussion of the isolation and identification of *Vibrio cholerae* and other enteropathogenic vibrios, refer to the procedures outlined in the references.

Results

After 18-24 hours of incubation at 35°C, sucrose-fermenting vibrios (*V. cholerae*, *V. alginolyticus*, *V. harveyi*, *V. cincinnatiensis*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, some *V. vulnificus*) appear as medium-sized, smooth, opaque, thin-edged yellow colonies on TCBS Agar.[6] The other clinically important vibrios and most *V. vulnificus* do not ferment sucrose and appear green.[6]

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. Further tests are necessary for identification and confirmation of *Vibrio* spp.[11]
3. On initial isolation, *V. parahaemolyticus* may be confused with *Aeromonas hydrophila*, *Plesiomonas shigelloides* and *Pseudomonas* species.[12]
4. Sucrose-fermenting *Proteus* species produce yellow colonies which may resemble those of *Vibrio*.[11]
5. TCBS is an unsatisfactory medium for oxidase testing of *Vibrio* spp.[13]
6. A few strains of *V. cholerae* may appear green or colorless on TCBS due to delayed sucrose fermentation.[11]

References

1. **Kobayashi, T., S. Enomoto, R. Sakazaki, and S. Kuwahara.** 1963. A new selective medium for pathogenic vibrios, TCBS (modified Nakanishi's agar). Jpn. J. Bacteriol. **18**:387.
2. **Nakanishi, Y.** 1963. An isolation agar medium for cholerae and enteropathogenic halophilic vibrios. Modern Media **9**:246.
3. **McLaughlin, J. C.** 1995. Vibrio, p. 465-476. *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.
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Packaging

TCBS Agar	100 g	0650-15
	500 g	0650-17

Bacto® m TEC Agar

Intended Use

Bacto m TEC Agar is used for isolating, differentiating and rapidly enumerating thermotolerant *Escherichia coli* from water by membrane filtration and an *in situ* urease test.

Also Known As

m TEC is an abbreviation for membrane (medium) Thermotolerant *E. coli*.

Summary and Explanation

Escherichia coli is widely used as an indicator of fecal pollution in water. There are many procedures for enumerating *E. coli* based on its

ability to grow at elevated temperatures and produce indole from tryptophane.[1,2] The determination of indole production in conjunction with the most-probable-number procedure often requires the use of another medium and additional incubation time.

The membrane filter procedure has been recognized by *Standard Methods for the Examination of Water and Wastewater* as an alternate test procedure.[3] In 1981, Dufour et al. developed a simple, accurate, nonlethal membrane filter technique for the rapid enumeration of *E. coli*.[4] This medium, m TEC Agar, quantifies *E. coli* within 24 hours without requiring subculture and identification of isolates. The authors reported that they were able to recover *E. coli* from marine, estuarine and fresh water samples.

Principles of the Procedure

m TEC Agar contains sufficient nutrients to support the growth of *E. coli*. Proteose peptone is a source of nitrogen, amino acids, carbon and amino acids. Yeast Extract provides trace elements, vitamins and amino acids. Potassium Phosphate Monobasic and Potassium Phosphate Dibasic offer buffering capabilities. Lactose is a fermentable carbohydrate and carbon source. Sodium Lauryl Sulfate and Sodium Desoxycholate are selective against gram-positive bacteria. Brom cresol purple and brom phenol red are indicator components and Bacto Agar solidifies the medium.

Formula

m TEC Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Sodium Chloride	7.5 g
Potassium Phosphate Monobasic	1 g
Potassium Phosphate Dibasic	3.3 g
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
Brom Cresol Purple	0.08 g
Brom Phenol Red	0.08 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

m TEC Agar

Materials Required But Not Provided

Autoclave
Sterile Petri dishes, 50 x 10 mm
Membrane filter equipment
Sterile 47 mm, 0.45 µm, gridded membrane filters
Pipettes
Stereoscopic microscope
Dilution blanks
35°C incubator
44.5°C waterbath or incubator
Waterproof plastic bags if water bath is used
Sterile absorbent pads
Urea
Phenol Red

User Quality Control

Identity Specifications

Dehydrated Appearance: Green to grayish tan, free-flowing and homogeneous.

Solution: 4.53% solution, soluble in distilled or deionized water on boiling. Solution is deep purple with red cast, slightly opalescent.

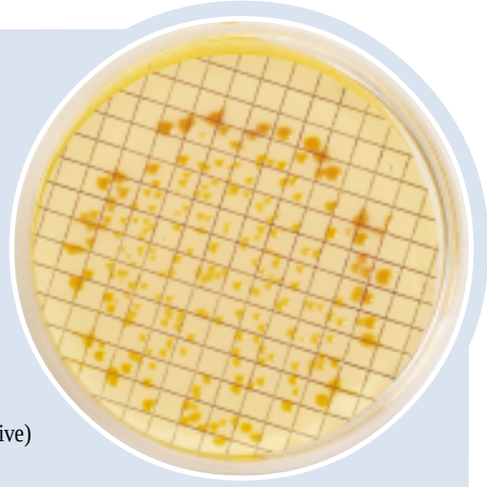
Reaction of 4.53% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Prepare m TEC Agar per label directions. Inoculate and incubate the plates at 35°C for two hours. Transfer plates and incubate at 44.5 ± 0.5°C for approximately 22 ± 2 hours. After incubation, filters are removed and placed over pads, saturated with approximately 2 ml of urease substrate. Yellow to yellow-brown colonies (urease negative) are counted after 15-20 minutes.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	APPEARANCE
Escherichia coli	8739	20-80	good	yellow to yellow-brown colonies

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



Escherichia coli ATCC® 8739

Method of Preparation

m TEC Agar

1. Suspend 45.3 g 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 4-5 mls amounts into 50 x 10 mm Petri dishes and allow to solidify.

Urea Substrate

1. Combine 2 g urea and 10 mg phenol red in 100 ml distilled water.
2. Adjust pH to 5.0 ± 0.2.
3. Store at 2-8°C. Use within one week.

Note: Other methods may recommend an alternative pH.[3,6] Prepare substrate according to recommended guidelines.

Specimen Collection and Preparation

Water samples should be collected and prepared in accordance to recommended guidelines.[3,6]

Test Procedure

1. Follow the membrane filter procedure described in *Standard Methods for the Examination of Water and Wastewater*.[3]
2. Incubate inoculated plates for 2 hours at 35°C to resuscitate injured cells.
3. Transfer the plates to a 44.5 ± 0.5°C waterbath or incubator and incubate for 22 ± 2 hours.
4. Transfer countable filters to pads saturated with urea substrate.
5. After 15-20 minutes, count all yellow to yellow-brown colonies with the aid of a stereoscopic microscope.

Results

Yellow to yellow-brown colonies (urease negative) may be presumptively identified as *E. coli*.

Limitations of the Procedure

1. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
2. The urease test is required to presumptively identify *E. coli*.
3. Choose a water sample size that will result in 20-80 colonies per filter. Plates containing more than 80 colonies are not recommended because high counts may not provide accurate urease test results.
4. Do not trap air bubbles underneath the filter.

References

1. **Mara, D. D.** 1973. A single medium for the rapid detection of *Escherichia coli* at 44°C. J. Hyg. **71**:783-785.
2. **Pugsley, A. P., L. J. Evision, and A. James.** 1973. A simple technique for the differentiation of *Escherichia coli* in water examination. Water RES. **7**:1431-1437.
3. **Eaton, A. D., L. S. 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.
4. **Dufour, A. P., E. R. Strickland, and V. J. Cabelli.** 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. **41**:1152- 1158.
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6. **1996 Annual Book of ASTM Standards**, Water and Environmental Technology (PCN: 01-110296-16). ASTM, WestConshohocken, PA.

Packaging

m TEC Agar	100 g	0334-15

Bacto® TPEY Agar Base

Intended Use

Bacto TPEY Agar Base is used with Bacto EY Tellurite Enrichment and Bacto Antimicrobic Vial P in detecting and enumerating coagulase-positive staphylococci.

Also Known As

TPEY Agar Base conforms with Tellurite-Polymyxin-Egg Yolk Agar Base.

Summary and Explanation

TPEY Agar Base is prepared according to the formulation of Crisley.[1,2] The complete medium is prepared by aseptically adding EY Tellurite Enrichment and Antimicrobic Vial P (polymyxin B) to the sterile TPEY Agar Base. This medium permits the isolation and enumeration of coagulase-positive staphylococci from a variety of specimens such as food products, air, dust and soil. Coagulase-negative staphylococci and other organisms are markedly to completely inhibited.

Principles of the Procedure

Lithium Chloride, Potassium Tellurite and Polymyxin B inhibit a wide variety of microorganisms, including coagulase-negative staphylococci. Yeast Extract provides vitamins and cofactors required for growth, as well as additional sources of nitrogen and carbon. Tryptone provides nitrogen, vitamins and amino acids. Mannitol is an energy source. Sodium Chloride maintains the osmotic balance. Bacto Agar is incorporated into the medium as a solidifying agent.

Formula

TPEY Agar Base

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Mannitol	5 g
Sodium Chloride	20 g
Lithium Chloride	2 g
Bacto Agar	18 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) MAY CAUSE HARM TO THE UNBORN CHILD. (US) 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 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

TPEY Agar Base
EY Tellurite Enrichment
Antimicrobic Vial P (Polymyxin B)

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	60 grams per 900 ml solution, soluble in distilled or deionized water on boiling. Prior to adding enrichment, solution is light to medium amber, opalescent.
Prepared Medium:	Yellowish-beige, opaque.
Reaction of 6.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY APPEARANCE	HALO†
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	–	–
Staphylococcus aureus	25923*	100-1,000	good	black	+
Staphylococcus epidermidis	14990	100-1,000	poor to fair	black	–

†Zone of precipitation/clearing around the colony.

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.

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 60 grams of TPEY Agar Base in 900 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Aseptically add 100 ml of EY Tellurite Enrichment warmed to room temperature and 10 ml of rehydrated Antimicrobic Vial P. Mix thoroughly.

   Alternatively, use 100 ml of a 30% egg yolk emulsion, 10 ml of Chapman Tellurite Solution 1% and 0.4 ml of filter sterilized 1% polymyxin B solution.
5. Pour 15-17 ml amounts into sterile Petri dishes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references.[3]

Results

Coagulase-positive staphylococci form black or dark-gray colonies due to the reduction of colorless tellurite to free tellurium. Three types of egg yolk precipitation reactions are produced by coagulase-positive staphylococci:

1. a discrete zone of precipitated egg yolk around and beneath the colonies;
2. a clear zone or halo surrounding the colonies with a possible zone of precipitate beneath the colonies; and,
3. no zone or halo around the colonies, but a precipitate beneath the colonies.

Limitations of the Procedure

1. Mannitol-positive and/or tellurite-positive staphylococcal strains that are coagulase-negative are occasionally found. Definitive identification of *S. aureus*, therefore, should be based primarily on the coagulase reaction, with mannitol fermentation and tellurite reduction being used only for confirmation.[3,5]
2. The prepared medium becomes less inhibitory to coagulase-negative strains of staphylococci if it is stored for longer than one week.[2]
3. Graves and Frazier[4] showed that *Bacillus* spp. able to grow on TPEY Agar produce an antibiotic that inhibits growth of staphylococci.

References

1. **Crisley, F. D., R. Angelotti, and M. J. Foter.** 1964. Multiplication of *Staphylococcus aureus* in synthetic cream fillings and pies. Public Health Rep. **79**:369.
2. **Crisley, F. D., J. T. Peeler, and R. Angelotti.** 1965. Comparative evaluation of five selective and differential media for the detection and enumeration of coagulase-positive staphylococci in foods. Appl. Microbiol. **13**:140.

3. **Koneman, E. W., S. D. Allen, V. R. Dowell, Jr., and H. M. Sommers.** 1979. Color atlas and textbook of diagnostic microbiology, 2nd ed. J. B. Lippincott Company, Philadelphia, PA.
4. **Graves, R. R., and W. C. Frazier.** 1963. Food microorganisms influencing the growth of *Staphylococcus aureus.* Appl. Microbiol. **11**:513.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, M.D.

Packaging

TPEY Agar Base	500 g	0556-17

Bacto® TSA Blood Agar Base · Bacto Tryptic Soy Blood Agar Base No. 2 · Bacto Tryptic Soy Blood Agar Base EH

Intended Use

Bacto TSA Blood Agar Base is used with blood in isolating and cultivating fastidious microorganisms where clear and distinct hemolytic reactions are of prime importance.

Bacto Tryptic Soy Blood Agar Base No. 2 is used with blood in isolating and cultivating fastidious microorganisms from specimens where clear and distinct hemolytic reactions are of prime importance.

Bacto Tryptic Soy Blood Agar Base EH is used with blood in isolating and cultivating fastidious microorganisms from specimens where clear and distinct hemolytic reactions are of prime importance.

User Quality Control

Identity Specifications

TSA Blood Agar Base

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

Solution: 4.0% solution, soluble in distilled or deionized water upon boiling, medium amber, slightly opalescent without significant precipitate.

Prepared Medium: With 5% sheep blood - bright medium red, opaque, firm.

Reaction of 4.0% Solution at 25°C: pH 7.3 ± 0.2

Tryptic Soy Blood Agar Base No. 2

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

Solution: 4.0% solution, soluble in distilled or deionized water on boiling, light to medium amber, slightly opalescent without significant precipitate.

Prepared Medium: With 5% sheep blood - bright cherry red, opaque.

Reaction of 4.0% Solution at 25°C: pH 7.3 ± 0.2

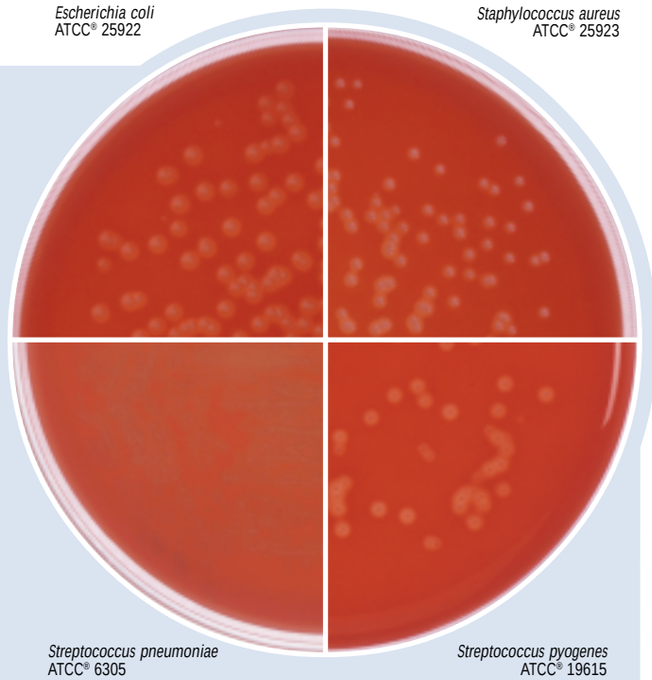
Tryptic Soy Blood Agar Base EH

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

Solution: 4.0% solution, soluble in distilled or deionized water on boiling, light to medium amber, clear to slightly opalescent without significant precipitate.

Prepared Medium: With 5% sheep blood - bright cherry red, opaque.

Reaction of 4.0% Solution at 25°C: pH 7.3 ± 0.2



Cultural Response

Prepare TSA Blood Agar per label directions. Inoculate and incubate at 35°C under 5-10% CO_2 for 18-48 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.

Also Known As

Blood Agar Base is abbreviated as BAB. Tryptic Soy Agar is abbreviated as TSA, and is referred to as Soybean-Casein Digest Agar Medium, USP.

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.

In 1919, Brown[1] experimented with blood agar formulations to determine their affects on colony formation and hemolysis. Growth of pneumococci was noticeably influenced when a medium contained peptone manufactured by Difco.

Tryptic Soy Agar is based on the Soybean-Casein Digest formula specified by US Pharmacopeia.[2] Tryptic Soy Agar is a general purpose medium used for multiple applications, e.g., maintaining culture collections, performing colony counts[3] and testing bacterial contaminants in cosmetics.[4] TSA Blood Agar Base was developed to achieve good growth and to improve hemolytic reactions of pathogenic microorganisms.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH represent further improvements to TSA Blood Agar Base. TSA Blood Agar Base No. 2 provides clearer hemolytic reactions with group A streptococci while TSA Blood Agar Base EH provides dramatic, enhanced hemolysis.

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

Principles of the Procedure

Blood Agar Base media formulations have been prepared using specially selected raw materials to support good growth of a wide variety of fastidious microorganisms. TSA Blood Agar Base contains two peptones, Pancreatic Digest of Casein and Papaic Digest of Soybean Meal, which provide nitrogen, carbon, amino acids and vitamins. Agar is a solidifying agent; Sodium Chloride maintains osmotic balance.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH are similar in composition to TSA Blood Agar Base. The formulations have been modified through the use of peptones (Tryptone H and Tryptone H Plus) developed at Difco Laboratories to improve and enhance hemolysin production while minimizing antagonism or loss in activity of streptococcal hemolysins. Both basal media contain Soytone for additional nitrogen, Agar as a solidifying agent, and Sodium Chloride to maintain osmotic balance.

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 basal medium used.[7]

Blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci.[8]

Formula

TSA Blood Agar Base

Formula Per Liter

Pancreatic Digest of Casein	15 g
Papaic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Tryptic Soy Blood Agar Base No. 2

Formula Per Liter

Bacto Tryptone H	15 g
Bacto Soytone	5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Tryptic Soy Blood Agar Base EH

Formula Per Liter

Bacto Tryptone H Plus	15 g
Bacto Soytone	5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

TSA Blood Agar Base
Tryptic Soy Blood Agar Base No. 2
Tryptic Soy Blood Agar Base EH

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:
   TSA Blood Agar Base - 40 grams;
   Tryptic Soy Blood Agar Base No. 2 - 40 grams;
   Tryptic Soy Blood Agar Base EH - 40 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. 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.[7]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.
3. Examine the medium for growth and hemolytic reactions after 18-24 and 48 hours incubation.
4. Four types of hemolysis on blood agar media have been described:[9]
   a. Alpha hemolysis (α) is the reduction of hemoglobin to meth emoglobin in the medium surrounding the colony, causing 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 hemoly sis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. TSA 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, immuno-logical 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.[7]
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.[7] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci, NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **The United States Pharmacopeia (USP XXIII) and The National Formulary (NF 18).** 1995. Sterility tests, p. 1686-1690. United States Pharmacopeial Convention Inc., Rockville, MD.
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9. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, p. 1.6.1-1.6.7, Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington D.C.
10. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. p. 415. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

TSA Blood Agar Base	500 g	0026-17
	2 kg	0026-07
	10 kg	0026-08
Tryptic Soy Blood Agar Base No. 2	500 g	0027-17
	2 kg	0027-07
	10 kg	0027-08
Tryptic Soy Blood Agar Base EH	500 g	0028-17
	2 kg	0028-07
	10 kg	0028-08

Bacto® TT Broth Base Hajna

Intended Use

Bacto TT Broth Base Hajna is used for enriching *Salmonella* from food and dairy products prior to isolation procedures.

Also Known As

TT Broth Base Hajna is also referred to as Tetrathionate Broth Base Hajna.

Summary and Explanation

TT Broth Base Hajna is used as a selective enrichment for the cultivation of *Salmonella* spp. *Salmonella* organisms can be injured in food-processing procedures. These procedures include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives and sanitizers.[1] Although injured cells may not form colonies on selective media, they can cause disease if ingested.[2] *Salmonella* spp., in particular, cause many types of infections from mild self-limiting gastroenteritis

to life-threatening typhoid fever.[3] The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting less than 7 days.[3]

TT Broth Base Hajna conforms to the formulation of Hajna and Damon.[4] The medium is a modification of the enrichment described by Kauffmann[5] and Knox.[6] Hajna and Damon[4] developed a new broth containing yeast extract, peptone, carbon sources and the selective agents, sodium desoxycholate and brilliant green (replacing Bile Salts).

TT Broth Base Hajna is used in testing *Salmonella* in egg processing plants.[7] It is specified in the Compendium of Methods for the Microbiological Examination of Foods.[8]

Principles of the Procedure

Tryptose provides nitrogen and amino acids. Yeast Extract supplies growth factors and vitamins. Dextrose and Mannitol are fermentable carbohydrates. Selectivity is accomplished by the combination of Sodium Thiosulfate and tetrathionate, suppressing coliform organisms.[6] Tetrathionate is formed in the medium by the addition of a solution containing iodine and potassium iodide. Organisms containing the enzyme tetrathionate reductase will proliferate in this medium.

Sodium Desoxycholate and Brilliant Green are selective agents that suppress coliform bacteria and inhibit gram-positive organisms. Sodium Chloride maintains the osmotic balance of the medium. Calcium Carbonate is a neutralizer that absorbs toxic metabolites.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige to very light green, free-flowing, homogeneous.
Solution:	9.15% solution, insoluble in distilled or deionized water on boiling; light green, slightly opalescent with a heavy white precipitate.
Prepared Medium:	Light green, slightly opalescent with a heavy white precipitate.
Reaction of 9.15% Solution at 25°C:	pH 7.6 ± 0.2 (after addition of the iodine solution)

Cultural Response

Prepare TT Broth Base Hajna with 4% iodine solution per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, plate the inoculated broth onto MacConkey Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR ON MACCONKEY AGAR
Escherichia coli	25922*	100-1,000	none to poor	pink with bile ppt.
Salmonella typhimurium	14028*	100-1,000	good	colorless

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

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

Formula

TT Broth Base Hajna

Formula Per Liter

Bacto Yeast Extract	2 g
Bacto Tryptose	18 g
Bacto Dextrose	0.5 g
Bacto D-Mannitol	2.5 g
Sodium Desoxycholate	0.5 g
Sodium Chloride	5 g
Sodium Thiosulfate	38 g
Calcium Carbonate	25 g
Brilliant Green	0.01 g

Final pH 7.6 ± 0.2 at 25°C

Precautions

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

   FIRST AID:

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

TT Broth Base Hajna

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Iodine crystals
Potassium iodide
MacConkey Agar

Method of Preparation

1. Suspend 91.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Cool below 50°C.
4. Add 40 ml iodine solution (5 grams iodine crystals and 8 grams potassium iodide dissolved in 40 ml distilled or deionized water) and mix well.

5. Dispense into sterile tubes while keeping suspension well mixed.
6. Do not heat the medium after adding iodine.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy. For a complete discussion on the collection, isolation and identification of *Salmonella*, refer to the appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for results.

References

1. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of salmonellae in foods. J. Food Prot. **44**:385-386.
2. **Sorrells, K. M., M. L. Speck, and J. A. Warren.** 1970. Pathogenicity of *Salmonella gallinarum* after metabolic injury by freezing. Appl. Microbiol. **19**:39- 43.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Hajna, A. A., and S. R. Damon.** 1956. New enrichment and plating medium for the isolation of *Salmonella* and *Shigella* organisms. Appl. Microbiol. **4**:341.
5. **Kauffman, F.** 1930. Ein kombiniertes Anreicherungsverfahren fur Typhus-und-Paratyphusbazillen. Zentralb. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abr. I Orig. **113**:148.
6. **Knox, R., P. H. Gell, and M. R. Pollack.** 1942. Selective media for organisms of the *Salmonella group*. J. Pathol. Bacteriol. **54**:469-483.
7. **Catalano, C. R., and S. J. Knable.** 1994. Incidence of *Salmonella* in Pennsylvania egg processing plants and destruction by high pH. J. Food Prot. **57**:587-591.
8. **Russell, S. F., J.-Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. *In* C. Vanderzant, and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

TT Broth Base Hajna	500 g	0491-17
	2 kg	0491-07

Bacto® Tellurite Blood Solution

Intended Use

Bacto Tellurite Blood Solution is used with Bacto Proteose No. 3 Agar and Bacto Dextrose in isolating *Corynebacterium diphtheriae*.

Summary and Explanation

Tellurite Blood Solution is used in the cultural diagnosis of diphtheria, an acute infectious disease primarily of the upper respiratory tract but occasionally of the skin.[1] Diphtheria is caused by toxigenic strains of *C. diphtheriae*, of which there are three biotypes; mitis, intermedius and gravis.[1] The symptoms of the disease are development of a pharyngeal membrane, sore throat, malaise, headache and nausea.[2] Death can result from respiratory obstruction by the membrane or from myocarditis caused by the toxin.[2]

Tellurite Blood Solution is a mixture of defibrinated bloods with added potassium tellurite. This solution is used to markedly reduce growth of commensal organisms encountered when isolating *Corynebacterium diphtheriae*.

User Quality Control

Identity Specifications

Solution Appearance: Dark, red-brown liquid.

Cultural Response

Prepare Proteose No. 3 Agar with Dextrose (1.5 grams per liter) and 5% Tellurite Blood Solution. Heat to 70-80°C to chocolatize the medium. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR
Corynebacterium diptheriae type *gravis*	8028	100-1,000	good	black
Corynebacterium diptheriae type *intermedius*	8032	100-1,000	good	black
Corynebacterium diptheriae type *mitis*	8024	100-1,000	good	black
Escherichia coli	25922*	1,000-2,000	inhibited	–
Streptococcus pyogenes	19616*	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.

Principles of the Procedure

Tellurite Blood Solution contains defibrinated blood for essential nutrients to enhance the growth of *C. diphtheriae*. Potassium Tellurite is a selective agent that inhibits the growth of commensal organisms.

Reagent

Tellurite Blood Solution is a combination of approximately 95% defibrinated, lysed horse and bovine blood from which most of the cellular debris has been removed. One-percent (1%) Potassium Tellurite is added.

Precautions

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

Storage

Store Tellurite Blood Solution 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

Tellurite Blood Solution

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

1. Shake Tellurite Blood Solution before use.
2. Refer to the final concentration of Tellurite Blood Solution in the formula of the medium being prepared. Add Tellurite Blood Solution as required.

Specimen Collection and Preparation[1]

Both throat and nasopharyngeal specimens are necessary in cases of respiratory illness. If cutaneous diphtheria is suspected, collect skin, throat and nasopharynx specimens. Use sterile silica gel for shipping clinical specimens when cultures are not taken locally.

Test Procedure

For a complete discussion of the collection, isolation and identification of *C. diphtheriae* and other *Corynebacterium* spp., refer to appropriate procedures in the references.[1,2]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Definitive identification of a strain of *C. diphtheriae* as a true pathogen requires demonstration of toxin production.[3]

References

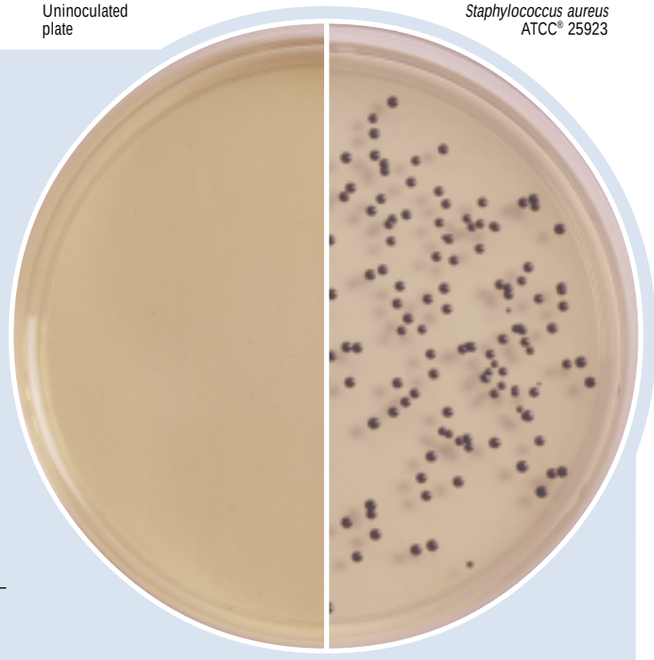
1. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
2. **Clarridge, J. E., and C. A. Spiegel.** 1995. *Corynebacterium* and miscellaneous irregular gram-positive rods, Erysipelothrix, and *Gardnerella*, p. 357-377. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Tellurite Blood Solution	6 x 25 ml	0139-66

Tellurite Glycine Agar

Bacto® Tellurite Glycine Agar · Bacto Chapman Tellurite Solution 1%



User Quality Control

Identity Specifications

Tellurite Glycine Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.25% solution; soluble in distilled or deionized water upon boiling. Medium amber, opalescent with precipitation.
Prepared Medium:	Medium amber, opalescent with precipitation.
Reaction of 6.25% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Tellurite Glycine Agar per label directions and enrich with Chapman Tellurite Solution 1%. Incubate inoculated medium at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	1,000-2,000	inhibited	–
Staphylococcus aureus	25923*	100-1,000	good	black
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.

Intended Use

Bacto Tellurite Glycine Agar is used with Bacto Chapman Tellurite Solution 1% for isolating coagulase-positive staphylococci.

Summary and Explanation

The coagulase-positive species *Staphylococcus aureus* is well documented as a human opportunistic pathogen.[3] Foods are examined for the presence of *S. aureus* and/or its enterotoxins to confirm that *S. aureus* is the causative agent of foodborne illness, to determine whether a food is the source of "staph" food poisoning, and to determine post-processing contamination.[4]

Ludlam[2] described a selective medium for the isolation of staphylococci. This medium was alkaline in reaction, contained mannitol, and lithium chloride with potassium tellurite as the selective agents. Zebovitz, Evans and Niven[1] modified Ludlam's medium by adding glycine as a selective agent and adjusting the reaction of the basal medium to pH 7.2 instead of pH 9.6.

Tellurite Glycine Agar is prepared according to the formula of Zebovitz, Evans and Niven.[1] The medium permits the isolation of coagulase-positive staphylococci from food, air, dust, soil and clinical specimens. Coagulase-negative staphylococci and other bacteria are markedly to completely inhibited.

Principles of the Procedure

Tryptone and Soytone are sources of nitrogen and amino acids in Tellurite Glycine Agar. Yeast Extract is a vitamin source in this formulation. D-Mannitol is a source of fermentable carbohydrate for coagulase-positive staphylococci. Lithium chloride, glycine and potassium tellurite are the selective agents. Dipotassium phosphate is used to buffer the medium. Bacto Agar is the solidifying agent.

Chapman Tellurite Solution is a sterile 1% solution of potassium tellurite, a differential agent. Coagulase-positive staphylococci reduce tellurite and produce black colonies.[5]

Formula

Tellurite Glycine Agar

Formula Per Liter

Bacto Yeast Extract	6.5 g
Bacto Soytone	3.5 g
Bacto Tryptone	10 g
Glycine	10 g
Bacto D-Manniol	5 g
Dipotassium Phosphate	5 g
Lithium Chloride	5 g
Bacto Agar	17.5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Tellurite Glycine Agar: 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.

   Chapman Tellurite Solution 1%: MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.(US) Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

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

Storage

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

Store Chapman Tellurite Solution 1% at 15-30°C.

Expiration Date

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

Procedure

Materials Provided

Bacto Tellurite Glycine Agar
Bacto Chapman Tellurite Solution 1%

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (50-55°C) (optional)
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. Aseptically add 10 ml Chapman Tellurite Solution 1% to the medium at 50-55°C. Mix well.
5. Dispense as desired.

Specimen Collection and Preparation

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

Test Procedure

For a complete discussion on the isolation and identification of coagulase-positive staphylococci from clinical specimens refer to appropriate procedures.[3,6] For the examination of staphylococci in foods refer to standard methods.[4,7]

Results

Coagulase-positive staphylococci produce black colonies within 24 hours of incubation at 35°C.

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. An occasional coagulase-negative staphylococci may produce small gray colonies, not readily confused with black coagulase-positive colonies.

References

1. **Zebovitz, E., J. B. Evans, and C. F. Niven Jr.** 1955. Tellurite glycine agar: A selective plating medium for the quantitative detection of coagulase-positive staphylococci. J. Bacteriol. **70**:686-690.
2. **Ludlam.** 1949. Monthly Bull. Ministry of Health. **8**:15.
3. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*, p. 282 - 298. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, M.D.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, M.D.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
7. **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

Tellurite Glycine Agar	500 g	0617-17
Chapman Tellurite Solution 1%	6 x 1 ml	0299-51
	6 x 25 ml	0299-66

Bacto® Tergitol 7 Agar
Bacto Tergitol 7 Broth

Intended Use

Bacto Tergitol 7 Agar and Bacto Tergitol 7 Broth are selective media used for enumerating and differentiating coliform bacteria.

Also Known As

Bacto Tergitol 7 Agar and Bacto Tergitol 7 Broth are also know as T7 Agar and T7 Broth, respectively.

Summary and Explanation

Tergitol 7 Agar and Broth, prepared according to the formula published by Chapman, are selective for *Escherichia coli* and members of the coliform group.[1] Chapman reported that the addition of Tergitol 7 to an agar medium consisting of Proteose Peptone No. 3, Yeast Extract, lactose, and brom thymol blue permitted unrestricted development of all coliform bacteria and inhibited development of gram-negative spore formers as well as gram-positive microorganisms. Counts of coliform organisms on Tergitol 7 Agar plates were found to be 30% higher than on some other selective media.

Chapman[2] modified his original Tergitol 7 Agar formula by adding 40 mg of triphenyltetrazolium chloride (TTC) per liter. This medium was found to be helpful in the early recognition and identification of *Escherichia coli*. Confirmation of the presence of *E. coli* was possible after only 10 hours incubation at 35°C. Chapman also reported that Tergitol 7 Agar with added TTC gave a selective medium suitable for the isolation of *Candida* and other fungi. *Candida* growing on this medium produce white, circular, convex, entire colonies about 1 mm in diameter in 24 hours. *Candida* colonies may appear pale blue because of the color of the medium, while yeasts produce red colonies.

Tergitol 7 Agar with TTC was shown to be useful in routine water analysis and the examination of foods.[3,4] The medium conforms with the recommendations of the APHA.[5]

Principles of the Procedure

Tergitol 7 (sodium heptadecyl sulfate) inhibits growth of gram-positive microorganisms and spore-forming gram-negative microorganisms, as well as the swarming of *Proteus*, while allowing for superior recovery of coliforms. Lactose fermentation is indicated by a color change of the pH indicator, brom thymol blue. Lactose-fermenting microorganisms produce yellow colonies. *Escherichia coli* produces yellow colonies with yellow zones, while *Enterobacter* and *Klebsiella* colonies are greenish-yellow. Nonfermenting organisms, such as *Salmonella* and *Shigella*, produce colonies surrounded by blue zones.

When TTC is added to the medium, it serves as an indicator of bacterial growth. TTC is rapidly reduced to insoluble red formazan by most lactose-fermenting organisms except *Escherichia coli*, *Enterobacter* and *Klebsiella* species. In the presence of TTC, lactose fermenters, which includes the coliforms, produce greenish-yellow colonies with yellow zones, while lactose nonfermenters produce red colonies surrounded by blue zones.

Proteose Peptone No. 3 provides 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. The Agar incorporated into Tergitol 7 Agar serves as a solidifying agent.

Formula

Tergitol 7 Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Bacto Agar	15 g
Tergitol 7	0.1 g
Bacto Brom Thymol Blue	0.025 g

Final pH 6.9 ± 0.2 at 25°C

Tergitol 7 Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Tergitol 7	0.1 g
Bacto Brom Thymol Blue	0.025 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 media below 30°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

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

User Quality Control

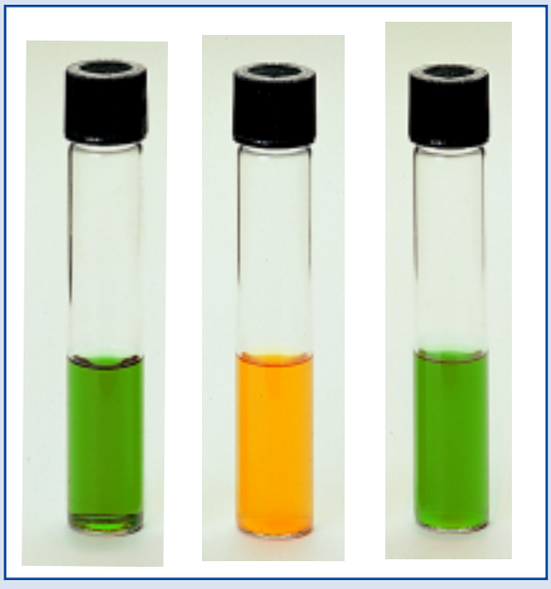
Identity Specifications

Tergitol 7 Agar

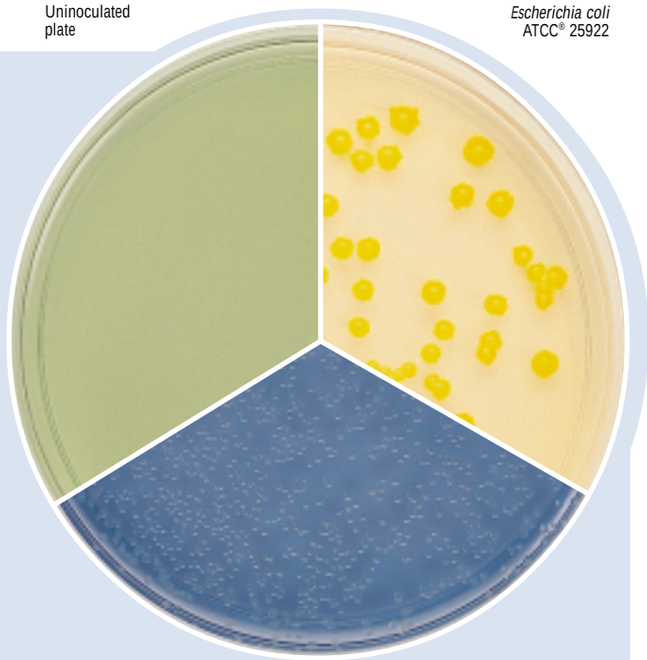
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in distilled or deionized water on boiling. Solution is green, slightly opalescent.
Prepared Plates:	Green, slightly opalescent without precipitate.
Reaction of 3.3% Solution at 25°C:	pH 6.9 ± 0.2

Tergitol 7 Broth

Dehydrated Appearance:	Beige, may have slight greenish tint, free-flowing, homogeneous.
Solution:	1.8% solution, soluble in distilled or deionized water on boiling.
Prepared Tubes:	Green, slightly opalescent.
Reaction of 1.8% Solution at 25°C:	pH 6.9 ± 0.2



Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Salmonella typhimurium* ATCC® 14028



Cultural Response

Prepare medium per label directions. Inoculate Tergitol 7 Agar plates with test organisms. Inoculate Tergitol 7 Broth tubes and leave caps loosened. Incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Enterococcus faecalis	19433	100-1,000	none to poor	N/A
Escherichia coli	25922*	100-1,000	good	+
Salmonella typhimurium	14028*	100-1,000	good	–

+ = positive, yellow colony or medium
– = negative, blue colony or medium as directed.

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

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

Procedure

Materials Provided

Tergitol 7 Agar
Tergitol 7 Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
OPTIONAL: Bacto TTC Solution 1% or Bacto TTC

Method of Preparation

1. Suspend medium in 1 liter distilled or deionized water:
   Tergitol 7 Agar-33 grams;
   Tergitol 7 Broth-18 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. OPTION: Cool Tergitol 7 Agar to 50°C. Add 4 ml of either TTC Solution 1% or a filter-sterilized 1% solution of TTC.

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. Since the medium with TTC permits growth of coliform organisms, this fact must be taken into consideration in the isolation of *Candida* from specimens.
2. Pour plates do not give satisfactory results.
3. Allow plates to dry with lids slightly ajar for 1-2 hours after dispensing.[6]
4. Reduction of TTC is an irreversible reaction that produces an insoluble formazan compound.

References

1. **Chapman, G. H.** 1947. A superior culture medium for the enumeration and differentiation of coliforms. J. Bacteriol. **53**:504.
2. **Chapman, G. H.** 1951. A culture medium for detecting and confirming *Escherichia coli* in ten hours. Am. J. Public Health **41**:1381.
3. **Kulp, W., C. Mascoli, and O. Tavshanjian.** 1953. Use of tergitol-7 triphenyl tetrazolium chloride agar as the coliform confirmatory medium in routine sanitary water analysis. Am. J. Public Health **43**:1111.
4. **Mossel, D. A. A.** 1962. An ecological investigation on the usefulness of two specific modifications of Eijkman's test as an element of the methods for the detecting of faecal contamination of foods. J. Appl. Bacteriol. **25**:20.
5. **Speck, Marvin L.** (ed.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Tergitol 7 Agar	500 g	0455-17
Tergitol 7 Broth	500 g	0912-17
TTC Solution 1%	30 ml	3112-67*

*Store at 2-8°C

Bacto® Terrific Broth

Intended Use

Bacto Terrific Broth is used with Bacto Glycerol in cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

Terrific Broth is a highly enriched medium developed by Tartoff and Hobbs to improve yield in plasmid bearing *E. coli*.[1] Recombinant strains have an extended growth phase in the medium. The addition of

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.76% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 4.76% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (C600)	23724	100-1,000	good
Escherichia coli (HB101)	33694	100-1,000	good
Escherichia coli (DH-1)	33849	100-1,000	good
Escherichia coli (JM103)	39403	100-1,000	good
Escherichia coli (JM107)	47014	100-1,000	good
Escherichia coli (DH-5)	53868	100-1,000	good

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

extra Tryptone and Yeast Extract in the medium allows higher plasmid yield per volume. Glycerol is used as the carbohydrate source in this formulation. Unlike glucose, glycerol is not fermented to acetic acid.

Principles of the Procedure

Tryptone and Yeast Extract provide necessary nutrients and cofactors for excellent growth of recombinant strains of *E. coli*. The Yeast Extract concentration is increased to allow for elevated cell yields. Potassium Phosphates are added to provide potassium for cellular systems and prevent cell death due to a drop in pH. Glycerol is added as a carbon and energy source.

Formula

Terrific Broth

Formula Per Liter

Bacto Tryptone	12 g
Bacto Yeast Extract	24 g
Potassium Phosphate, Dibasic	9.4 g
Potassium Phosphate, Monobasic	2.2 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 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

Terrific Broth

Materials Required But Not Provided

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

Method of Preparation

1. Dissolve 47.6 grams in 1 liter of distilled or deionized water. Add 4 ml of Glycerol to the medium.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[1,2]

Results

Growth is evident in the form of turbidity.

References

1. **Tartoff, K. D., and C. A. Hobbs**. 1987. Improved media for growing plasmid and cosmid clones. Bethesda Research Laboratories Focus **9:**12.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis**. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Packaging

Terrific Broth	500 g	0438-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Tetrathionate Broth Base

Intended Use

Bacto Tetrathionate Broth Base is used for enriching *Salmonella* species during isolation procedures.

Also Known As

Tetrathionate Broth (Base) can be abbreviated as TT Broth (Base).

Summary and Explanation

Tetrathionate Broth Base is used as a selective enrichment for the cultivation of *Salmonella* species that may be present in small numbers and compete with intestinal flora. *Salmonella* organisms may also be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives and sanitizers.[1] Although injured cells may not form colonies on selective media, they can, if ingested, cause disease.[2] *Salmonella* species cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.[3] 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.[3]

Mueller[4] demonstrated the effectiveness of Tetrathionate Broth for enriching typhoid and paratyphoid bacilli while inhibiting coliform organisms. Using modified Mueller's broth, Kauffmann[5,6] increased the number of positive isolates. Tetrathionate Broth was used in studies for the poultry industry[7,8] and in a collaborative study for rapid screening of *Salmonella* in food.[9]

Modifications of Tetrathionate Broth Base include TT Broth w/Brilliant Green, TT Broth Base, Hajna, Mueller Kauffmann Tetrathionate Broth Base and Tetrathionate with Novobiocin.[10]

Tetrathionate Broth Base is specified in standard methods[12,13,14,15] for *Salmonella* testing. Tetrathionate Broth is used in processing fecal cultures for bacteria.[16]

Principles of the Procedure

Proteose Peptone provides the nitrogen, carbon, vitamins and amino acids in Tetrathionate Broth Base. Selectivity is accomplished by the combination of Sodium Thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.[17] (Tetrathionate is formed in the medium upon addition of the iodine and potassium iodide solution.) Organisms containing the enzyme tetrathionate reductase will proliferate in the medium. Bile Salts, a selective agent, suppresses coliform bacteria and inhibits gram-positive organisms. Calcium Carbonate neutralizes and absorbs toxic metabolites.

Formula

Tetrathionate Broth Base

Formula Per Liter

Bacto Proteose Peptone	5 g
Bacto Bile Salts	1 g
Sodium Thiosulfate	30 g
Calcium Carbonate	10 g

Final pH 8.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Tetrathionate Broth Base:**

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

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	White to off-white, may have a slight greenish tint, free-flowing, homogeneous.
Solution:	4.6% solution, insoluble in distilled or deionized water. Suspension is milky white, opaque. On standing, supernatant is nearly colorless to light yellow over a heavy white precipitate.
Prepared Medium:	Nearly colorless to light yellow supernatant over a heavy white precipitate.
Reaction of 4.6% Solution at 25°C:	pH 8.4 ± 0.2 (measured before iodine solution is added).

Cultural Response

Prepare Tetrathionate Broth Base per label directions and enrich with 2% iodine solution. Inoculate with 100-1,000 CFUs of test organism and incubate at 35 ± 2°C for 18-24 hours. Subculture onto MacConkey Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH	COLOR OF COLONY ON MACCONKEY AGAR
Escherichia coli	25922*	little or no increase in the number of colonies	pink w/bile precipitate
Salmonella typhimurium	14028*	good	colorless

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

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

Storage

Store Tetrathionate Broth Base dehydrated 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

Tetrathionate Broth Base

Materials Required But Not Provided

Glassware
Distilled or deionized water
Iodine solution (see Method of Preparation)
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile tubes

Method of Preparation

1. Suspend 4.6 grams in 100 ml distilled or deionized water.
2. Heat to boiling. DO NOT AUTOCLAVE.
3. Cool to below 60°C.
4. Add 2 ml iodine solution (6 grams iodine crystals and 5 grams potassium iodide in 20 ml water).
5. DO NOT REHEAT MEDIUM. DO NOT AUTOCLAVE.
6. Dispense into sterile tubes. Use immediately.

Specimen Collection and Preparation

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

Test Procedure

For a complete discussion of the isolation and identification of *Salmonella*, refer to appropriate procedures outlined in the references.

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. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of salmonellae in foods. J. Food Prot. **44**:385-386.
2. **Sorrells, K. M., M. L. Speck, and J. A. Warren.** 1970. Pathogenicity of *Salmonella gallinarum* after metabolic injury by freezing. Appl. Microbiol. **19**:39- 43.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Muller, L.** 1923. Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. C. R. Soc. Biol. **89**:434. Paris.
5. **Kauffmann, F.** 1930. Ein kombiniertes anreicherungsverfahren fur typhus-und-paratyphusbacillen. Zentralb. Bakteriol. Parasitenke. Infektionskr. Hyg. Abt.I orig. **113**:148.
6. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren fur Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
7. **Jones, F. T., R. C. Axtell, D. V. Rives, S. E. Scheideler, F. R. Tarver, Jr., R. L. Walker, and M. J. Wineland.** 1991. A survey of *Salmonella* contamination in modern broiler production. J. Food Prot. **54**:502-507.
8. **Barnhart, H. M., D. W. Dressen, R. Bastien, and O. C. Pancorbo.** 1991. Prevalence of *Salmonella enteritidis* and other serovars in ovaries of layer hens at time of slaughter. J. Food Prot. **54**:488-492.
9. **Eckner, K. F., W. A. Dustman, M. S. Curiale, R. S. Flowers, and B. J. Robison.** 1994. Elevated-temperature, colorimetric, monoclonal, enzyme-linked immunosorbent assay for rapid screening of *Salmonella* in foods: collaborative study. J. Assoc. Off. Anal. Chem. **77**:374-383.
10. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 751-754, Williams & Wilkins, Baltimore, MD.
11. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*. p 5.01-5.20. *In* Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, MD.
12. **Russell, S. F., J.-Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p.371-422. *In* Vanderzant, C. and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
13. **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.
14. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
15. **Federal Register.** 1991. Animal and plant health inspection service: chicken affected by *Salmonella enteritidis*, final rule. Fed. Regist. **56**:3730-3743.
16. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D. C.
17. **Knox, R., P. H. Gell, and M. R. Pollack.** 1942. Selective media for organisms of the *Salmonella* group. J. Pathol. Bacteriol. **54**:469-483.

Packaging

Tetrathionate Broth Base	500 g	0104-17
	2 kg	0104-07

Bacto® m Tetrathionate Broth Base

Intended Use

Bacto m Tetrathionate Broth Base is used for selectively enriching *Salmonella* by membrane filtration prior to isolation procedures.

Summary and Explanation

Salmonella spp. cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.[2] 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.[2]

Tetrathionate Broth, in single strength and without calcium carbonate, was used by Kabler and Clark[1] for the preliminary enrichment of *Salmonella* other than *S. typhi*. Their investigation found that approximately 80% of *Salmonella* species recovered were from mixed cultures and that most coliforms were suppressed. The presence of calcium carbonate in the medium gave poor, erratic results. The authors[1] reported favorable results for enrichment of *S. typhimurium* in the membrane filtration technique. This study used a 3-hour preliminary incubation on pads saturated with Tetrathionate Broth followed by 15 hours incubation on m Brilliant Green Broth.

m Tetrathionate Broth Base has the same formulation as Tetrathionate Broth Base, except that calcium carbonate has been omitted.[1]

Principles of the Procedure

Proteose Peptone provides nitrogen, vitamins, amino acids and carbon in m Tetrathionate Broth Base. Selectivity is achieved by the combination of sodium thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.[3] Tetrathionate is formed in the medium by the addition of iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will proliferate in the medium. Bile Salts, a selective agent, is added to suppress coliform bacteria and inhibit gram-positive organisms.

Formula

m Tetrathionate Broth Base

Formula Per Liter

Bacto Proteose Peptone	5 g
Bacto Bile Salts	1 g
Sodium Thiosulfate	30 g

Final pH 8.0 ± 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): 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 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 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

m Tetrathionate Broth Base

Materials Required But Not Provided

Glassware

Membrane filtration equipment
Iodine solution (see Method of Preparation, #4)
Incubator (35°C)
Sterile Petri dishes, 50 x 9 mm
m Brilliant Green Broth
Distilled or deionized water

Method of Preparation

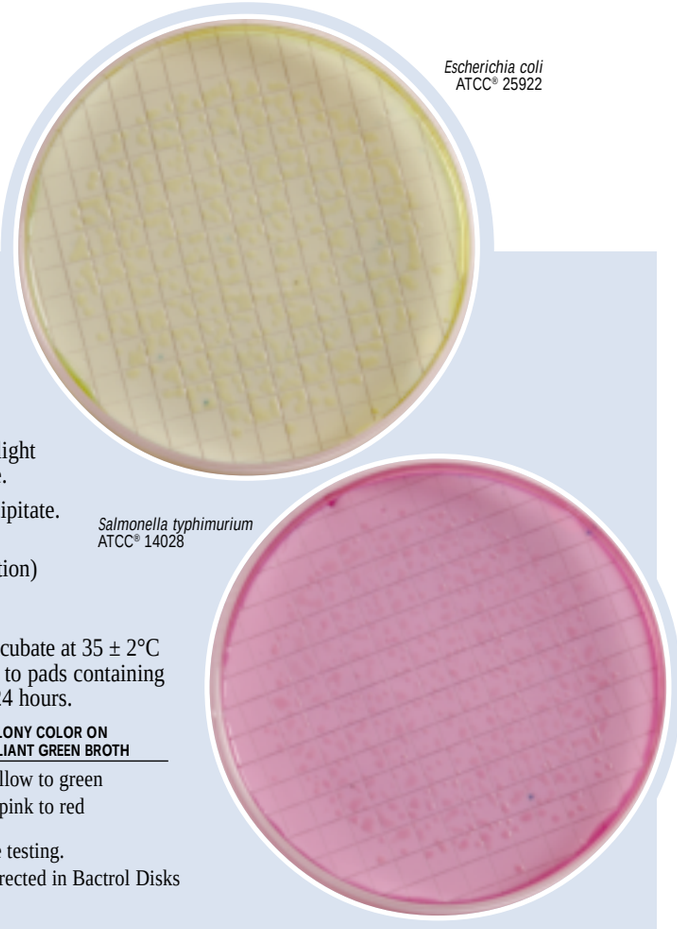
1. Suspend 3.6 grams in 100 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Cool medium to below 60°C.
4. Add 2 ml iodine solution (6 grams iodine crystals and 5 grams potassium iodide dissolved in 20 ml distilled or deionized water). Use the complete medium containing iodine within 24 hours.
5. Do not heat the medium after adding the iodine solution.
6. Dispense 2 ml amounts of medium onto sterile absorbent pads in 50-60 mm Petri dishes.

Specimen Collection and Preparation

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

Test Procedure

1. Perform membrane filtration with the inoculum to be tested.



Escherichia coli
ATCC® 25922

Salmonella typhimurium
ATCC® 14028

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige with greenish cast, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear, may have some precipitate.
Prepared Medium:	Light amber, clear, may have some precipitate.
Reaction of 3.6% Solution at 25°C:	pH 8.0 ± 0.2 (before adding iodine solution)

Cultural Response

Prepare m Tetrathionate Broth per label directions. Inoculate and incubate at 35 ± 2°C in a humid atmosphere for approximately 3 hours. Transfer filters to pads containing m Brilliant Green Broth and continue incubation to a total of 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR ON m BRILLIANT GREEN BROTH
Escherichia coli	25922*	30-300	good	yellow to green
Salmonella typhimurium	14028*	30-300	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.

2. Place the membrane filter on a pad soaked with m Tetrathionate Broth (with iodine) and incubate at 35 ± 2°C for 3 hours.
3. Aseptically transfer the filter to a pad soaked with 2 ml m Brilliant Green Broth in a 50-60 mm Petri dish.
4. Incubate at 35 ± 2°C for an additional 15-21 hours (total incubation to 18-24 hours) in a humid atmosphere.

Results

Examine for growth. *Salmonella* species produce 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. **Kabler and Clark.** 1952. Am. J. Public Health **42**:390.
2. **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.
3. **Knox, R., P. H. Gell, and M. R. Pollack.** 1942. Selective media for organisms of the *Salmonella* group. J. Pathol. Bacteriol. **54**:469-483.

Packaging

m Tetrathionate Broth Base	500 g	0580-17

Bacto® Thermoacidurans Agar

Intended Use

Bacto Thermoacidurans Agar is used for isolating and cultivating *Bacillus coagulans* (*Bacillus thermoacidurans*) from foods.

Summary and Explanation

Stern et al.[1] described a medium for isolating *B. coagulans* (*B. thermoacidurans*) which causes "flat sour" spoilage in tomato juice. "Flat sour" spoilage of canned foods can caused by *Bacillus coagulans* (*Bacillus thermoacidurans*). Bacterial growth results in a 0.3-0.5 drop in pH, while the ends of the can remain flat. *B. coagulans* is a soil microorganism that can be found in canned tomato products and dairy products. Conditions favorable to multiplication of the organism can result in spoilage of the food product.[2]

Thermoacidurans agar can also be used to isolate mesophilic spore forming anaerobes (*Clostridium* spp.) from foods.[2] These microorganisms tolerate high heat, grow in the absence of oxygen and grow over the range of temperatures used in canned and processed foods. They are of primary importance in spoilage of low-acid foods packed in hermetically sealed containers.[2]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in distilled or deionized water on boiling; light amber, opalescent without significant precipitate.
Prepared Medium:	Light amber, opalescent without precipitate.
Reaction of 3.9% Solution at 25°C:	pH 5.0 ± 0.2

Cultural Response

Prepare Thermoacidurans Agar per label instructions. Inoculate and incubate the plates at 55 ± 1°C for 18-48 hours.

TEST ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus coagulans	7050	100-1,000	good

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

Principles of the Procedure

Thermoacidurans Agar contains Proteose Peptone to provide the carbon and nitrogen for general growth requirements. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Bacto Agar is a solidifying agent.

Formula

Formula Per Liter

Bacto Thermoacidurans Agar

Bacto Yeast Extract	5 g
Bacto Proteose Peptone	5 g
Bacto Dextrose	5 g
Dipotassium Phosphate	4 g
Bacto Agar	20 g

Final pH 5.0 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Bacto Thermoacidurans Agar

Materials Required but not Provided

Glassware
Distilled or deionized water

Autoclave
Incubator (55°C)
Petri dishes

Method of Preparation

1. Suspend 39 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.
4. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[1,2]

Results

Growth is evident in the form of turbidity.

Limitations of the Procedure

Microorganisms other than *B. coagulans* may grow on this medium. Perform microscopic examination and biochemical tests to identify to genus and species if necessary.

References

1. **Stern, Hegarty, and Williams.** 1942. Food Research **7**:186.
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Thermoacidurans Agar	500 g	0303-17

Bacto® Thiamine Assay Medium
Bacto Thiamine Assay Medium LV

Intended Use

Bacto Thiamine Assay Medium is used for determining thiamine concentration by the microbiological assay technique.

Bacto Thiamine Assay Medium LV is used for determining thiamine concentration by the microbiological assay technique using *Lactobacillus viridescens* ATCC® 12706.

Also Known As

Thiamine is also know as Vitamin B1.

Summary and Explanation

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

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

Thiamine Assay Medium is prepared according to the formula by Sarett and Cheldelin.[1] *Lactobacillus fermentum* ATCC® 9338 is used as the test organism in the microbiological assay of thiamine.

Thiamine Assay Medium LV, patterned after APT medium, was described by Deibel, Evans and Niven[2] for the microbiological assay of thiamine using *Lactobacillus viridescens* ATCC® 12706.

Nutritional studies by Evans and Niven[3] on the heterofermentative lactobacilli that cause greening in cured meat products indicated that thiamine was an essential vitamin for growth of these organisms. Deibel, Evans and Niven[4] described APT medium for lactobacilli cultivation. They reported that lactobacilli required at least 10 ng thiamine per ml for growth in contrast to 0.2 to 3 ng per ml for thiamine-requiring streptococci, leuconostocs and staphylococci. Further, they suggested that lactobacilli requiring large amounts of thiamine might be employed in microbiological assay procedures. In 1957,[2] these authors described a medium for the microbiological assay of thiamine using *Lactobacillus viridescens* ATCC® 12706 as the test organism. This medium is known as Thiamine Assay Medium LV.

Principles of the Procedure

Thiamine Assay Medium and Thiamine Assay Medium LV are free from thiamine, but contain all other nutrients and vitamins essential for the growth of the test organisms. The addition of thiamine in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Thiamine Assay Medium

Formula Per Liter		
Thiamine-Free Tryptone	22	g
Bacto Vitamin Assay Casamino Acids	5	g
Bacto Dextrose	40	g
Sodium Acetate	15	g
L-Cystine	0.2	g
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Riboflavin	200	µg
Calcium Pantothenate	200	µg
Niacin	200	µg
Pyridoxine Hydrochloride	200	µg
p-Aminobenzoic Acid	200	µg
Folic Acid	5	µg
Biotin	0.8	µg
Dipotassium Phosphate	1	g
Monopotassium Phosphate	1	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20	mg
Ferrous Sulfate	20	mg
Manganese Sulfate	20	mg

Final pH 6.5 ± 0.2 at 25°C

Thiamine Assay Medium LV

Formula Per Liter

Thiamine-Free Yeast Extract	10 g
Thiamine-Free Tryptone	20 g
Bacto Dextrose	20 g
Sodium Citrate	10 g
Dipotassium Phosphate	10 g
Sodium Chloride	10 g
Magnesium Sulfate	1.6 g
Manganese Sulfate	0.28 g
Ferrous Sulfate	0.08 g
Tween 80	2 g

Final pH 6.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Thiamine Assay Medium

Dehydrated Medium:	Beige, homogeneous, tendency to clump.
Solution:	4.25% (single strength) and 8.5% (double strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. 4.25% solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	4.25% solution is light amber, clear, may have a slight precipitate.
Reaction of 4.25% Solution at 25°C:	pH 6.5 ± 0.2

Thiamine Assay Medium LV

Dehydrated Appearance:	Beige, homogeneous, tendency to clump.
Solution:	4.2% (single strength) and 8.4% (double strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. 4.2% solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	4.2% solution is light amber, clear, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Thiamine Assay Medium

Prepare single-strength Thiamine Assay Medium per label directions. Prepare a standard curve using a thiamine hydrochloride reference standard at 0.0 to 0.05 µg per 10 ml. Inoculate with *Lactobacillus fermentum* ATCC® 9338 and incubate with caps loosened at 35-37°C for 16-18 hours. Read percent transmittance using a spectrophotometer at 660 nm.

Thiamine Assay Medium LV

Prepare single-strength Thiamine Assay Medium LV per label directions. Prepare a standard curve using a thiamine hydrochloride reference standard at 0.0 to 25.0 µg per 10 ml. Inoculate with *Lactobacillus viridescens* ATCC® 12706 and incubate with caps loosened at 30 ± 2°C for 16-20 hours. Read percent transmittance using a spectrophotometer at 660 nm.

Precautions

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

Thiamine Assay Medium
Thiamine Assay Medium LV

Materials Required But Not Provided

Glassware
Autoclave
Spectrophotometer or nephelometer
Centrifuge
Incubator, 30°C and 35°C
Sterile tubes and caps
Sterile 0.85% NaCl
Stock culture of *Lactobacillus viridescens* ATCC® 12706 or
Stock culture of *Lactobacillus fermentum* ATCC® 9338
Lactobacilli Agar AOAC
Micro Assay Culture Agar
APT Agar
Lactobacilli Broth AOAC
Micro Inoculum Broth
APT Broth
Thiamine hydrochloride

Method of Preparation

1. Suspend the medium in 100 ml distilled or deionized water:
   Thiamine Assay Medium - 8.5 grams;
   Thiamine Assay Medium LV - 8.4 grams.
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

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

Test Procedure

Thiamine Assay Medium

Prepare stock cultures of the test organism, *Lactobacillus fermentum* ATCC® 9338, by stab inoculation on Lactobacilli Agar AOAC or Micro Assay Culture Agar. After 24-48 hours incubation at 35-37°C, keep the tubes in the refrigerator. Make transfers in triplicate at monthly intervals.

Prepare the inoculum by subculturing a stock culture of the test organism in 10 ml of Lactobacilli Broth AOAC or Micro Inoculum Broth. After 16-18 hours incubation at 35-37°C, centrifuge the cells under aseptic conditions and decant the supernatant liquid. Wash the cells three times with 10 ml sterile 0.85% NaCl. After the third wash, resuspend the cells in 10 ml sterile 0.85% NaCI. Add 0.5 ml of this suspension to 100 ml sterile 0.85% NaCl. Use one drop of the resulting suspension to inoculate the assay tubes.

A standard curve should be run with each assay because conditions of heating and incubation temperature that influence the standard curve readings cannot always be duplicated.

The tubes for the Thiamine Assay Medium standard curve contain 0.0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04 and 0.05 µg of thiamine hydrochloride per 10 ml tube. The most effective assay range for Thiamine Assay Medium is between 0.005 and 0.03 µg thiamine.

Prepare the stock solution of thiamine required for the preparation of the standard curve in Thiamine Assay Medium as follows:

1. Dissolve 0.1 gram of thiamine hydrochloride in 1,000 ml of distilled water (100 µg/ml).
2. Add 1 ml of the solution in Step 1 to 99 ml distilled water (1 µg/ml).
3. Add 1 ml of the solution in Step 2 to 99 ml distilled water to give a final concentration of 10 ng (0.010 µg/ml). Use 0.0, 0.5, 1, 1.5, 2, 3, 4 and 5 ml of this final solution per tube. Prepare fresh stock solution daily.

After 20-24 hours incubation at 35-37°C, *L. fermentum* ATCC® 9338 is capable of using the pyrimidine and thiazole moieties of the thiamine molecule. It is essential that the growth response be measured turbidimetrically prior to this time. Incubate the tubes at 35-37°C for 16-18 hours, then place in the refrigerator for 15-30 minutes to stop growth. The growth can then be measured by any suitable nephelometric method.

Thiamine Assay Medium LV

Prepare stock cultures of the test organism, *L. viridescens* ATCC® 12706, by stab inoculation on APT Agar or Lactobacilli Agar AOAC. After 24-48 hours incubation at 30 ± 2°C, keep the tubes in the refrigerator. Make transfers in triplicate at monthly intervals.

Prepare the inoculum by subculturing a stock culture of the test organism to 10 ml APT Broth or Lactobacilli Broth AOAC. After 16-20 hours incubation at 30 ± 2°C, centrifuge the cells under aseptic conditions and decant the supernatant liquid. Wash the cells three times with 10 ml sterile 0.85% NaCl. After the third wash, resuspend the cells in 10 ml sterile 0.85% NaCl. Add 1 ml of this cell suspension to 100 ml sterile 0.85% NaCl. Use one drop of this suspension to inoculate the assay tubes.

A standard curve should be run with each assay because conditions of heating and incubation temperature that influence the standard curve readings cannot always be duplicated.

The standard curve for Thiamine Assay Medium LV is obtained by using thiamine at levels of 0.0, 1, 2.5, 5, 7.5, 10, 15, 20 and 25 ng of thiamine hydrochloride per 10 ml tube. This is obtained by using 0.0, 0.2, 0.5, 1, 1.5, 2, 3, 4 and 5 ml of the standard solution, which contains 5 ng (0.005 µg) thiamine hydrochloride per ml. The most effective assay range is between 2.5 and 20 ng per tube.

The solution for preparing the standard curve for Thiamine Assay Medium LV may be prepared as follows:

1. Dissolve 50 mg of thiamine hydrochloride in 500 ml distilled water (100 µg/ml).
2. Add 1 ml of the solution in Step 1 to 99 ml distilled water (1 µg/ml).
3. Add 1 ml of the solution in Step 2 to 199 ml distilled water to give a final concentration of 5 ng (0.005 µg) per ml.

Following incubation of *L. viridescens* ATCC® 12706 at 30 ± 2°C for 16-20 hours, the growth response is measured turbidimetrically.

Results

Thiamine Assay Medium and Thiamine Assay Medium LV

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

Limitations of the Procedure

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

References

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Packaging

Thiamine Assay Medium	100 g	0326-15
Thiamine Assay Medium LV	100 g	0808-15

Thioglycollate Media

Bacto® Fluid Thioglycollate Medium · Bacto NIH Thioglycollate Broth · Bacto Brewer Thioglycollate Medium · Bacto Fluid Thioglycollate Medium w/Beef Extract · Bacto Fluid Thioglycollate Medium w/K Agar · Bacto Thioglycollate Medium w/o Dextrose · Bacto Thioglycollate Medium w/o Dextrose or Indicator · Bacto Thioglycollate Medium w/o Indicator

Intended Use

Bacto Fluid Thioglycollate Medium is used for detecting microorganisms in normally sterile materials. Fluid Thioglycollate Medium conforms to the formula specified by the US Pharmacopeia XXIII (USP)[1], the Code of Federal Regulations (21 CFR)[2] and European Pharmacopeia for sterility testing of pharmaceutical products, biologics and devices.

Bacto NIH Thioglycollate Broth is used in detecting microorganisms in normally sterile, turbid or viscous materials. This formula conforms with USP Alternate Thioglycollate Medium.[1]

Bacto Brewer Thioglycollate Medium is for detecting microorganisms in normally sterile materials.

Bacto Fluid Thioglycollate Medium w/Beef Extract is used in cultivating microorganisms from normally sterile biological products.

Bacto Fluid Thioglycollate Medium w/K Agar is for detecting microorganisms in normally sterile materials containing mercurial preservatives when clarity and early visualization of growth are desired.

Bacto Thioglycollate Medium w/o Dextrose and **Thioglycollate Medium w/o Dextrose or Indicator** are used for detecting microorganisms in normally sterile materials, especially those containing mercurial preservatives. These media formulations may be used with added carbohydrates for fermentation studies.

Bacto Thioglycollate Medium w/o Indicator is for detecting microorganisms in normally sterile materials, especially those containing mercurial preservatives, when no oxidation-reduction indicator is required.

Also Known As

Fluid Thioglycollate Medium is often referred to as Thioglycollate Medium and abbreviated as FTM.

NIH Thioglycollate Medium is also known as USP Thioglycollate Medium Alternative and Alternate Fluid Thioglycollate.

Thioglycollate Medium w/o Indicator and Thioglycollate Medium w/o Dextrose or Indicator have been called Thioglycollate Fermentation Media.

Summary and Explanation

Quastel and Stephenson[4] found that the presence of a small amount of a compound containing an -SH group (cysteine, thioglycollic acid, glutathione) permitted "aerobic" growth of *Clostridium sporogenes* in tryptic digest broth.

Falk, Bucca and Simmons[5] pointed out the advantages of using small quantities of agar (0.06-0.25%) in detecting contaminants during sterility testing of biologicals. The value of combining a small amount of agar and a reducing substance was demonstrated by Brewer.[6] Brewer's experiments revealed that in a liquid medium containing 0.05% agar, anaerobes grew equally well in the presence or absence of sodium thioglycollate. Marshall, Gunnish and Luxen[7] reported satisfactory cultivation of anaerobes in Brewer's Thioglycollate Medium in the presence of a mercurial preservative. Nungester, Hood and Warren[8] and Portwood[9] confirmed the neutralization of the bacteriostatic effect of mercurial compounds by sodium thioglycollate. Malin and Finn[10] reported the commonly used medium containing thioglycollate is inhibitory to some organisms in the presence of a carbohydrate. In 1941, the National Institutes of Health specified the use of two thioglycollate media in sterility testing, the Brewer Formula and the Linden Formula.[11] The Linden Formula was later referred to as Modified Brewer Thioglycollate Medium in which meat infusion was replaced by plant (soy) peptones.[12]

Fluid Thioglycollate Medium is prepared according to the formula in the FDA Bacteriological Analytical Manual (BAM)[13] and AOAC Official Methods of Analysis[14] for the examination of food, and for determining the phenol coefficient and sporicidal effects of disinfectants. Fluid Thioglycollate Medium is also specified for sterility checks on banked blood.[15]

Fluid Thioglycollate Medium w/ Beef Extract is recommended by Animal and Plant Health Inspection Service, USDA,[3] in the detection of viable bacteria in live vaccines. Thioglycollate Medium w/o Dextrose and Thioglycollate Medium w/o Dextrose or Indicator may be used with added carbohydrates for fermentation studies.

Thioglycollate Medium w/o Indicator is the medium of choice for diagnostic work because the lack of indicator avoids possible toxicity to organisms.[13] This medium supports a minimal inoculum with early visibility of growth.

When used as an enrichment broth to support plated media, thioglycollate media are often supplemented with hemin and vitamin K_1.[17] Several modifications of this medium, usually with the addition

User Quality Control

Identity Specifications

Fluid Thioglycollate Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.98% solution, soluble in distilled or deionized water on boiling. Appearance of solution immediately after sterilization - light amber, clear.
Prepared Medium:	Appearance of solution immediately after sterilization - light amber, clear. After cooling to room temperature, light amber, slightly opalescent with pink upper layer. If pink layer is greater than 10% of the tube, the medium may be restored once by heating on a steambath until the pink color disappears.
Reaction of 2.98% Solution at 25°C:	pH 7.1 ± 0.2

Brewer Thioglycollate Medium

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.05 % solution, soluble in distilled or deionized water on boiling; medium amber, clear to very slightly opalescent with upper 10% or less medium green on standing.
Prepared Medium:	Medium amber, clear to very slightly opalescent with upper 10% or less medium green.
Reaction of 4.05% Solution at 25°C:	pH 7.2 ± 0.2

NIH Thioglycollate Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in distilled or deionized water on boiling; light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.9% Solution at 25°C:	pH 7.1 ± 0.2

Fluid Thioglycollate Medium w/Beef Extract

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.47% solution, soluble upon boiling for 1-2 minutes. Immediately after sterilization, appearance is medium to dark amber, clear, becoming medium to dark amber with upper 10% or less of medium pink and slightly opalescent.
Prepared Medium:	Medium to dark amber with upper 10% or less medium pink, slightly opalescent.
Reaction of 3.47% Solution at 25°C:	pH 7.2 ± 0.2

Fluid Thioglycollate Medium w/K Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in distilled or deionized water upon boiling. Appearance of solution immediately after sterilization-light amber, clear. After cooling to room temperature and shaking, solution becomes pink with slight opalescence.
Prepared Medium:	Appearance of solution immediately after sterilization - light amber, clear. After cooling to room temperature, light amber, with some opalescence with upper 10% or less of medium pink.
Reaction of 2.9% Solution at 25°C:	pH 7.2 ± 0.2

Thioglycollate Medium w/o Dextrose

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in distilled or deionized water on boiling; light amber, clear to very slightly opalescent. Upper 10% or less medium green on standing.
Prepared Medium:	Light amber, very slightly opalescent with upper 10% or less medium turning green on cooling.
Reaction of 2.4% Solution at 25°C:	pH 7.2 ± 0.2

Thioglycollate Medium w/o Indicator

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in distilled or deionized water upon boiling. Appearance of solution immediately after sterilization-light amber, clear without significant precipitate. After cooling to room temperature, solution may exhibit slight opalescence.
Prepared Medium:	Appearance of solution immediately after sterilization - light amber, clear without significant precipitate. After cooling to room temperature, solution may exhibit slight opalescence.
Reaction of 2.9% Solution at 25°C:	pH 7.2 ± 0.2

Thioglycollate Medium w/o Dextrose or Indicator

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in distilled or deionized water upon boiling. Appearance of solution immediately after sterilization - light amber, clear with no significant precipitate. After cooling to room temperature - light, very slightly to slightly opalescent with no significant precipitate.
Prepared Medium:	Immediately after sterilization-light, clear with no significant precipitate. After cooling to room temperature - light, very slightly to slightly opalescent with no significant precipitate.
Reaction of 2.4% Solution at 25°C:	pH 7.2 ± 0.2

continued on following page

of sodium polyanetholesulfonate (SPS), are used in the inoculum of blood cultures specifically, for the isolation of anaerobes.[18]

The methodologies for the multiple applications using thioglycollate medium are outlined in the references.

Principles of the Procedure

Thioglycollate media support the growth of a large variety of fastidious microorganisms having a wide range of growth requirements. The nitrogen source, provided by Casitone, Infusion from Beef, Proteose Peptone, Beef Extract, Pancreatic Digest of Casein varies with the formula. Yeast Extract is added as a source of vitamins.

Sodium Thioglycollate, Thioglycollic Acid and L-Cystine lower the oxidation-reduction potential of the medium by removing oxygen to maintain a low Eh. By creating an environment with a low Eh, the reducing agents prevent the accumulation of peroxides which can be toxic to some organisms. The sulfhydryl groups (-SH) of these compounds also neutralize the antibacterial effect of mercurial preservatives, making thioglycollate media useful in testing material which contains heavy metals.

Resazurin or Methylene Blue are oxidation indicators. In the oxidized state, methylene blue appears green, resazurin turns pink. In the reduced state both compounds are colorless. Bacto Agar eliminates the need for seals because it retards dispersion of CO_2, diffusion of oxygen and reducing substances.[12] Substituting K Agar and Potassium Chloride for Bacto Agar and Sodium Chloride in Fluid Thioglycollate Medium w/K Agar produces a medium with greater clarity to facilitate earlier visual recognition of growth.

Dextrose is included in the formulations because many organisms show earlier and more vigorous growth. Sodium chloride is used to maintain the osmotic balance of the media. Potassium Chloride and Dipotassium Phosphate are used as buffering agents.

User Quality Control cont.

Cultural Response

Brewer Thioglycollate Medium (0236), **Thioglycollate Medium w/o Indicator** (0430), **Thioglycollate Medium w/o Dextrose** (0363), **Thioglycollate Medium w/o Dextrose or Indicator** (0432).

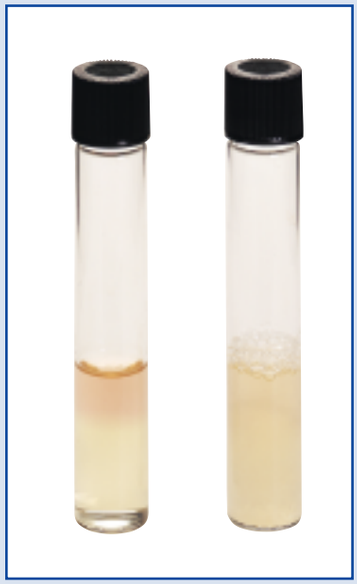
Medium was prepared per label directions. Tubes were inoculated with the test organisms and incubated at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Staphylococcus aureus	25923	10-100	good
Clostridium novyi	7659	10-100	good
Clostridium sporogenes	11437	10-100	good
Bacteroides fragilis	25285	10-100	good

Fluid Thioglycollate Medium w/K Agar (0607), **Fluid Thioglycollate Medium w/Beef Extract** (0697), **NIH Thioglycollate Broth** (0257).

Medium was prepared per label directions. Tubes were inoculated with the test organisms and incubated at 30-35°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis	6633	10-100	good
Bacteroides vulgatus	8482	10-100	good
Candida albicans	10231	10-100	good
Clostridium sporogenes	19404	10-100	good



Uninoculated tube — *Bacteroides vulgatus* ATCC® 8482

USP and EP Growth Promotion Procedure[1]

Fluid Thioglycollate Medium. Prepare FTM per label directions. Inoculum of 10-100 CFU were used and incubated for up to 7 days at temperature specified.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	INC. TEMP
Bacillus subtilis	6633*	10-100	growth must be evident	30-35°C
Bacteroides vulgatus	8482*	10-100	growth must be evident	30-35°C
Candida albicans	10231*	10-100	growth must be evident	20-25°C
Candida albicans	2091*	10-100	growth must be evident	20-25°C
Clostridium sporogenes	19404*	10-100	growth must be evident	30-35°C
Staphylococcus aureus	6538P*	10-100	good	30-35°C
Staphylococcus aureus	25923	10-100	good	30-35°C
Clostridium novyi	7659	10-100	good	30-35°C
Clostridium perfringes	13124	10-100	good	30-35°C

*Pharmacopeia growth promotion

Mercurial Neutralization

This test is performed by recovering the test organisms in Fluid Thioglycollate Medium after exposure to 1% Merthiolate®

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Staphylococcus aureus	6538P	1,000	good
Streptococcus pyogenes	19615**	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.

Formula

Fluid Thioglycollate Medium

Formula Per Liter

Bacto Casitone	15 g
Bacto Yeast Extract	5 g
Bacto Dextrose	5.5 g
Sodium Chloride	2.5 g
L-Cystine	0.5 g
Sodium Thioglycollate	0.5 g
Bacto Agar	0.75 g
Resazurin	0.001 g

Final pH 7.1 ± 0.2 at 25ºC

Thioglycollate Medium w/o Dextrose

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Casitone	15 g
L-Cystine	0.25 g
Sodium Chloride	2.5 g
Sodium Thioglycollate	0.5 ml
Bacto Agar	0.75 g
Methylene Blue	0.002 g

Final pH 7.2 ± 0.2 at 25ºC

Brewer Thioglycollate Medium

Formula Per Liter

Beef, Infusion from	500 g
Sodium Chloride	5 g
Dipotassium Phosphate	2 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	5 g
Sodium Thioglycollate	0.5 g
Bacto Agar	0.5 g
Methylene Blue	0.002 g

Final pH 7.2 ± 0.2 at 25ºC

NIH Thioglycollate Broth

Formula Per Liter

Bacto Casitone	15 g
Bacto Yeast Extract	5 g
Bacto Dextrose	5.5 g
Sodium Chloride	2.5 g
L-Cystine	0.5 g
Sodium Thioglycollate	0.5 g

Final pH 7.1 ± 0.2 at 25ºC

Fluid Thioglycollate Medium w/K Agar

Formula per liter

Bacto Casitone	15 g
Bacto Yeast Extract	5 g
Bacto Dextrose	5 g
Potassium Chloride	2.5 g
L-Cystine	0.5 g
Sodium Thioglycollate	0.5 ml
K Agar	0.45 g
Resazurin	0.001 g

Final pH 7.2 ± 0.2 at 25ºC

Thioglycollate Medium w/o Indicator

Formula per liter

Bacto Yeast Extract	5 g
Bacto Casitone	15 g
Bacto Dextrose	5 g
L-Cystine	0.25 g
Sodium Chloride	2.5 g
Sodium Thioglycollate	0.5 g
Bacto Agar	0.75 g

Final pH 7.2 ± 0.2 at 25ºC

Fluid Thioglycollate Medium w/Beef Extract

Formula Per Liter

Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Pancreatic Digest of Casein	15 g
Bacto Dextrose	5.5 g
Sodium Chloride	2.5 g
L-Cystine	0.5 g
Sodium Thioglycollate	0.5 g
Bacto Agar	0.75 g
Resazurin	0.001 g

Final pH 7.2 ± 0.2 at 25ºC

Thioglycollate Medium w/o Dextrose or Indicator

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Casitone	15 g
L-Cystine	0.25 g
Sodium Chloride	2.5 g
Sodium Thioglycollate	0.5 ml
Bacto Agar	0.75 g

Final pH 7.2 ± 0.2 at 25ºC

Precautions

1. For Laboratory Use:
   Fluid Thioglycollate Medium
   NIH Thioglycollate Broth
   Fluid Thioglycollate Medium w/K Agar
   Brewer Thioglycollate Medium
   Fluid Thioglycollate Medium w/Beef Extract
   Thioglycollate Medium w/o Dextrose
   Thioglycollate Medium w/o Indicator
   Thioglycollate Medium w/o Dextrose or Indicator
2. Do not reheat the media more than once, because continued reheating gives rise to toxicity. Do not to reheat **NIH Thioglycollate Broth**.
3. When testing human serum, treat all specimens as infectious agents.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

1. Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.
2. Store prepared media at 15-30°C.

3. For **Fluid Thioglycollate Medium, Fluid Thioglycollate Medium w/Beef Extract** and **Fluid Thioglycollate Medium w/K Agar,** if more than 30% of the medium is pink prior to use, reheat once (100°C) to drive off absorbed oxygen.

   For **Brewer Thioglycollate Medium**, if more than 20% of the medium is green prior to use, reheat once (100°C).

   After prolonged storage, reheat **Thioglycollate Medium w/o Indicator, Thioglycollate Medium w/o Dextrose or Indicator** and **Thioglycollate Medium w/o Dextrose** only once in flowing steam or a boiling water bath to drive off dissolved gases.

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

Fluid Thioglycollate Medium
NIH Thioglycollate Broth
Brewer Thioglycollate Medium
Fluid Thioglycollate Medium w/Beef Extract
Fluid Thioglycollate Medium w/K Agar
Thioglycollate Medium w/o Dextrose
Thioglycollate Medium w/o Dextrose or Indicator
Thioglycollate Medium w/o Indicator

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath
Sterile tubes

Method of Preparation

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

Fluid Thioglycollate Medium	29.8 g
Brewer Thioglycollate Medium	40.5 g
NIH Thioglycollate Broth	29 g
Fluid Thioglycollate Medium w/Beef Extract	34.7 g
Fluid Thioglycollate Medium w/K Agar	29 g
Thioglycollate Medium w/o Dextrose	24 g
Thioglycollate Medium w/o Indicator	29 g
Thioglycollate Medium w/o Dextrose or Indicator	24 g

2. Heat to boiling to dissolve completely. Avoid overheating.
3. Dispense as desired, using only clean, rust-free equipment.
4. Autoclave at 121°C for 15 minutes. Cool to room temperature.

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 bacteria and yeasts, refer to appropriate procedures outlined in the references.

Results

Typically growth is visually observed in the media. Gram negative bacilli tend to grow diffusely, gram positive cocci exhibit puff-ball type growth and strict aerobes, such as pseudomonads and yeast, tend to grow in a thin layer on the surface of the broth.

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Packaging

Fluid Thioglycollate Medium	100 g	0256-15
	500 g	0256-17
	2 kg	0256-07
	10 kg	0256-08
NIH Thioglycollate Broth	500 g	0257-17
Brewer Thioglycollate Medium	500 g	0236-17
	10 kg	0236-08
Fluid Thioglycollate Medium w/ Beef Extract	500 g	0697-17
	10 kg	0697-08
Fluid Thioglycollate Medium w/K Agar	500 g	0607-17
	2 kg	0607-07
Thioglycollate Medium w/o Dextrose	500 g	0363-17
Thioglycollate Medium w/o Dextrose or Indicator	500 g	0432-17
Thioglycollate Medium w/o Indicator	500 g	0430-17

Bacto® Thiol Medium Bacto Thiol Broth

Intended Use

Bacto Thiol Medium is used for cultivating organisms from body fluids and other materials containing penicillin, streptomycin or sulfonamides.

Bacto Thiol Broth is used for cultivating organisms from body fluids and other materials containing penicillin, streptomycin or sulfonamides.

Summary and Explanation

While studying *Vibrio fetus* cultivation, Huddleson[1] found that vibrios remained viable in Thiol Medium at room temperature for at least 150 days without transfer. Christensen[2] tested Thiol Medium for the ability to neutralize penicillin and streptomycin. Ten milliliters (10 ml) of Thiol Medium can inactivate up to 100 units of penicillin and up to 1,000 micrograms of streptomycin, producing luxuriant growth of staphylococci and other organisms from dilute inocula in 24 hours.

Szawatkowski[3] and Shanson and Barnicoat[4] reported Thiol Broth to be superior in supporting the growth of *Bacteroides* species in blood cultures. Thiol Broth was used to study the optimum incubation period of blood culture broths.[5] Media containing thiol and thioglycollate are recommended for recovery of nutritionally variant streptococci (NVS).[6]

Thiol Broth has the same formulation as Thiol Medium, omitting the agar. Thiol is cited in Clinical Microbiology Procedures Handbook[7] as a medium specific for anaerobic bacteria in blood cultures.

Principles of the Procedure

Proteose Peptone No. 3 and Yeast Extract provide nitrogen, vitamins and amino acids in Thiol media. Dextrose is a carbon source. Sodium Chloride maintains osmotic balance. Para-aminobenzoic Acid is a preservative. Thiol Complex is rich in sulfhydryl (-SH) groups, which neutralize the bacteriostatic and bactericidal effects of penicillin, streptomycin and sulfonamides. Thiol Medium contains 0.1% Bacto Agar to maintain an Eh potential that facilities anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment.[8]

Formula

Thiol Medium

Formula Per Liter

Bacto Proteose Peptone No.3	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	1 g
Sodium Chloride	5 g
Thiol Complex	8 g
Bacto Agar	1 g
p-Aminobenzoic Acid	0.05 g

Final pH 7.1 ± 0.2 at 25°C

Thiol Broth

Formula Per Liter

Bacto Proteose Peptone No.3	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	1 g
Sodium Chloride	5 g
Thiol Complex	8 g
p-Aminobenzoic Acid	0.05 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Use Thiol media within four days of preparation.

Procedure

Materials Provided

Thiol Medium
Thiol Broth

Materials Required But Not Provided

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

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Thiol Medium - 30 grams;
   Thiol Broth - 29 grams.
2. Heat to boiling to dissolve completely.
3. Dispense as desired, using clean, rust-free equipment to prevent precipitate formation.
4. Autoclave at 121°C for 15 minutes. Cool to room temperature.

User Quality Control

Identity Specifications

Thiol Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3% solution, soluble in distilled or deionized water on boiling. Very light to light amber, clear to very slightly opalescent when hot, opalescent after cooling.
Prepared Medium:	Very light amber; very slightly to slightly opalescent when hot, opalescent after cooling.
Reaction of 3% Solution at 25°C:	pH 7.1 ± 0.2

Thiol Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in distilled or deionized water on boiling; very light to light amber, clear to slightly opalescent.
Prepared Medium:	Very light amber, clear to slightly opalescent.
Reaction of 2.9% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare Thiol Medium and Thiol Broth per label directions. Test 5 unit, 100 unit and 1,000 unit concentrations of penicillin and 100 µg, 1,000 µg and 10,000 µg concentrations of streptomycin. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o ANTIBIOTICS	GROWTH w/ANTIBIOTICS
Staphylococcus aureus	25923*	100-1,000	good	good†
Streptococcus pyogenes	19615*	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.

†Antibiotic concentrations up to 100 units of penicillin or 1000 µg of streptomycin.

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 processing and interpretation of blood cultures and body fluids from clinical specimens, refer to appropriate references.[7,9]

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. Strict reliance on blood culture bottles containing Thiol Broth is not recommended for aerobic microorganisms. Always use an aerobic medium, for example, a vented Tryptic Soy Broth, for optimum isolation of the broad spectrum of microorganisms that can cause bacteremia or septicemia.

References

1. **Huddleson, I. F.** 1948. A satisfactory medium for the isolation, cultivation, and maintenance of viability of *Vibrio fetus* (bovine). J. Bacteriol. **56**:508.
2. **Christensen, C. W.** 1947. Presented at the Michigan Branch, Society of American Bacteriologists, Detroit, MI., December 12, 1947.
3. **Szawatkowski, M. V.** 1976. A comparison of three readily available types of anaerobic blood culture media. Med. Lab. Sci. **33**:5-12.
4. **Shanson, D. C., and M. Barnicoat.** 1975. An experimental comparison of Thiol broth with Brewer's thioglycollate for anaerobic blood cultures. J. Clin. Pathol. **28**:407-409.
5. **Murray, P. R.** 1985. Determination of the optimum incubation period of blood culture broths for the detection of clinically significant septicemia. J. Clin. Microbiol. **21**:481-485.
6. **Donnelly, J. P.** 1994. Nutritionally variant streptococci and B_6. Infect. Dis. Alert **6**:109-112.
7. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
8. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 802-804. Williams & Wilkins, Baltimore, MD.
9. **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

Thiol Medium	500 g	0307-17
Thiol Broth	500 g	0434-17
	10 kg	0434-08

Tinsdale Agar

Bacto® Tinsdale Base · Bacto Tinsdale Enrichment Desiccated

Intended Use

Bacto Tinsdale Base is used with Bacto Tinsdale Enrichment Desiccated in isolating and differentiating *Corynebacterium diphtheriae*.

Also Known As

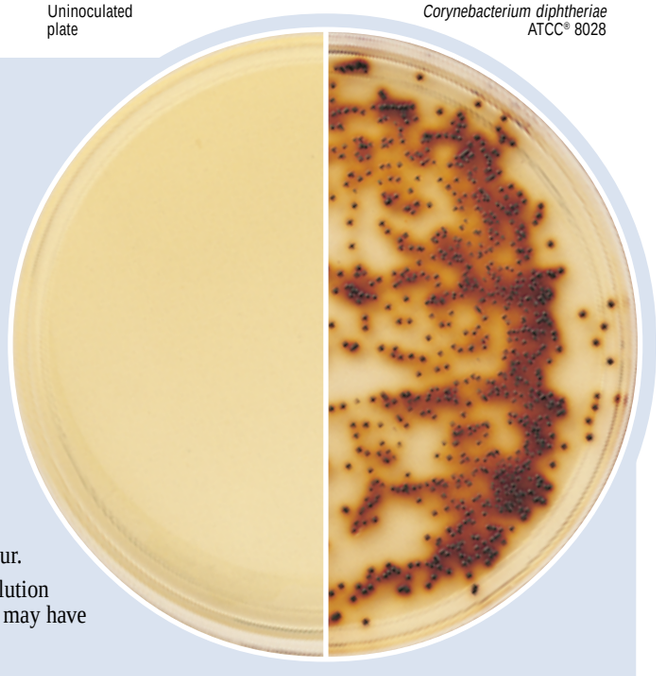
Tinsdale Base (TIN) is also called Tinsdale Selective Medium, Tinsdale Tellurite Medium and Tellurite Agar.

Summary and Explanation

Tinsdale Base, supplemented with Tinsdale Enrichment, is employed in the cultural diagnosis of diphtheria. Diphtheria, an acute infectious disease primarily of the upper respiratory tract but occasionally of the skin,[1] is caused by toxigenic strains of *Corynebacterium diphtheriae*. The three biotypes are mitis, intermedius and gravis.[1] The signs and symptoms of the disease are a pharyngeal membrane, sore throat, malaise, headache and nausea.[2] Death can result from respiratory obstruction by the membrane or myocarditis caused by the toxin.[2]

Tinsdale[3] developed a serum-cystine-thiosulfate-tellurite agar medium for the primary isolation and differentiation of *C. diphtheriae*. This formulation distinguished between *C. diphtheriae* and diphtheroids which exhibited similar characteristics. The differential principle is based on the capacity of *C. diphtheriae* to produce a brown or black halo around the colonies.

Billings[4] simplified Tinsdale Basal Medium by using Proteose Peptone No. 3 as a nutrient source. This modification improved the differential qualities and recovery of *Corynebacterium diphtheriae*. Tinsdale Base and Tinsdale Enrichment are prepared according to the Billings[4] modification. Moore and Parsons[5] confirmed the halo formation of *C. diphtheriae* with one exception; *C. ulcerans* occasionally produced colonies similar to *C. diphtheriae* and required biochemical identification.



User Quality Control

Identity Specifications

Tinsdale Base

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	4.5% solution, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, slightly opalescent to opalescent, without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent to opalescent without precipitate.
Reaction of 4.5% Solution at 25°C:	pH 7.4 ± 0.2

Tinsdale Enrichment Desiccated

Lyophilized Appearance:	Light to dark tan cake; variations may occur.
Solution:	Soluble in distilled or deionized water. Solution is light to dark amber, clear to opalescent, may have a slight precipitate.

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Corynebacterium diphtheriae type *gravis*	8028	100-1,000	good	brown with halos
Corynebacterium diphtheriae type *mitis*	8024	100-1,000	good	brown with halos
Klebsiella pneumoniae	13883*	100-1,000	marked to complete inhibition	–
Streptococcus pyogenes	19615*	100-1,000	fair	brown to black without halos

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.

Tinsdale Enrichment Desiccated contains Bovine Serum, Sodium Hydroxide, L-Cystine, Sodium Thiosulfate and Potassium Tellurite in the quantity and proportion described by Billings.[4]

Principles of the Procedure

Proteose Peptone No. 3 provides the nitrogen, vitamins, carbon and amino acids in Tinsdale Base. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent.

Tinsdale Enrichment contains Bovine Serum, which provides essential growth factors. Sodium Hydroxide maintains the pH. L-Cystine and Sodium Thiosulfate are H_2S indicators. Potassium Tellurite is a selective agent. The formation of black to brown halos surrounding the colony result from the reduction of potassium tellurite to metallic tellurite.

Stabbing the medium with an inoculating needle accentuates darkening of the medium by *C. diphtheriae*.

Formula

Tinsdale Base

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Sodium Chloride	5 g
Bacto Agar	20 g

pH 7.4 ± 0.2 at 25°C

Tinsdale Enrichment Desiccated

Contains Bovine Serum, L-Cystine, Sodium Hydroxide, Sodium Thiosulfate and Potassium Tellurite at pH 8.0-10.0 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 Tinsdale Enrichment Desiccated 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

Tinsdale Base
Tinsdale Enrichment Desiccated

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 45 grams of Tinsdale Base in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 100 ml amounts into flasks.
4. Autoclave at 121°C for 15 minutes.
5. Rehydrate Tinsdale Enrichment with 15 ml sterile distilled or deionized water and rotate in an end-over-end motion to dissolve completely.
6. Aseptically add 15 ml rehydrated Tinsdale Enrichment to each 100 ml of Tinsdale Base at 50-55°C. Mix well.
7. Dispense into sterile Petri dishes.

Specimen Collection and Preparation[1]

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

Test Procedure

1. For a complete discussion on the collection, isolation and identification of *Corynebacterium diphtheriae* and other *Corynebacterium* species, refer to the appropriate procedures outlined in the references.
2. Inoculate plates with the test organisms in a manner to obtain discrete colonies and stab the medium several times with an inoculating needle.
3. Definitive identification of a strain of *C. diphtheriae* as a true pathogen requires demonstration of toxin production.[6] Characteristic colonies of *C. diphtheriae* may be inoculated directly onto KL Virulence Agar enriched with KL Virulence Enrichment and containing KL Antitoxin Strips for toxigenicity tests.

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. Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C. diphtheriae*.[1]
3. *Corynebacterium ulcerans*, *Corynebacterium pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar.[1]
4. Do not read Tinsdale Agar early because several organisms may exhibit slight browning on this medium in 18 hours.[1]
5. Incubation at 5-10% CO_2 retards the development of halos on Tinsdale Agar.[1]
6. On media containing tellurite, diphtheria bacilli are shorter and stain more uniformly; however, granules are less readily observed than when grown on Loeffler's medium.[7]
7. Further biochemical tests may be necessary to distinguish between *C. diphtheriae* and *C. ulcerans* due to similar reactions on this medium.

References

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

2. **Clarridge, J. E., and C. A. Spiegel.** 1995. Corynebacterium and miscellaneous irregular gram-positive rod, *Erysipelothrix*, and *Gardnerella*, p. 357-377. *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. **Tinsdale, G. F. W.** 1947. A new medium for the isolation and identification of *C. diphtheriae* based on the production of hydrogen sulphide. J. Pathol. Bacteriol. **59**:461-466.
4. **Billings, E.** 1956. An investigation of Tinsdale Tellurite medium: its usefulness and mechanisms of halo-formation. M.S. thesis. University of Michigan, Ann Arbor, MI.
5. **Moore, M. S., and E. I. Parsons.** 1958. A study of a modified Tinsdale's medium for the primary isolation of *Corynebacterium diphtheriae*. J. Infect. Dis. **102**:88.
6. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
7. **Bailey, R. W., and E. G. Scott.** 1966. Diagnostic microbiology, 2nd ed., p. 213. The C. V. Mosby Company, St. Louis, MO.

Packaging

Tinsdale Base	500 g	0786-17
Tinsdale Enrichment Desiccated	6 x 15 ml	0342-33

Bacto® Todd Hewitt Broth

Intended Use

Bacto Todd Hewitt Broth is used for cultivating streptococci, pneumococci and other fastidious organisms; for cultivating group A streptococci prior to serological typing.

Also Known As

Todd Hewitt Broth can be abbreviated as THB.

Summary and Explanation

Todd Hewitt Broth was originally developed for the production of antigenic streptococcal hemolysin.[1] Todd Hewitt Broth is prepared according to the formula described by Updyke and Nickle[2] who compared media for type specific extract production of group A streptococci. This study was performed using Todd Hewitt Broth prepared with infusion of fresh beef heart as a control. Results showed Todd Hewitt Broth was particularly satisfactory for growth of group A streptococci for serological typing.

Elliott[3] reported that Todd Hewitt Broth prepared with neopeptone was excellent for growing group A streptococci for the production of type specific M substance. This is possible because proteinase is not produced in this medium.

Moody, et al.[4] used Todd Hewitt Broth in the fluorescent-antibody identification of group A streptococci from throat cultures. Todd Hewitt Broth is recommended as an enrichment medium for the growth of streptococcal cells in the identification of groups A and B by IF staining.[5] Todd Hewitt Broth was used as an enrichment broth for group A streptococci in a comparison study of a rapid antigen test.[6]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in distilled or deionized water; light to medium amber, clear with no significant precipitate.
Prepared Medium:	Light to medium amber, clear with no significant precipitate.
Reaction of 3.0% Solution at 25°C:	pH 7.8 ± 0.2

Cultural Response

Prepare Todd Hewitt Broth per label directions. Incubate inoculated tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good
Streptococcus pneumoniae	6303*	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.

Principles of the Procedures

Infusion from Beef Heart and Neopeptone provides the nitrogen, vitamins and amino acids in Todd Hewitt Broth. Dextrose is the carbon source, and a stimulant for hemolysin production.[7] Disodium Phosphate and Sodium Carbonate act as buffers to aid in neutralizing acid production from dextrose fermentation, and protect hemolysin from inactivation.[7] Sodium Chloride maintains the osmotic balance of the medium.

Formula

Todd Hewitt Broth

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Neopeptone	20 g
Bacto Dextrose	2 g
Sodium Chloride	2 g
Disodium Phosphate	0.4 g
Sodium Carbonate	2.5 g

Final pH 7.8 ± 0.2 at 25°C

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

Todd Hewitt Broth

Materials Required But Not Provided

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

Method of Preparation

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

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, identification and serological procedures of fastidious microorganisms, refer to the procedures described in appropriate references.[4,5,8,9]

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. Todd Hewitt Broth cannot be used unbuffered for bile solubility testing[10]

References

1. **Todd, E. W., and L. F. Hewitt**. 1932. A new culture medium for the production of antigenic streptococcal haemolysin. J. Pathol. Bacteriol. **35**:973.
2. **Updyke, E. L., and M. I. Nickle.** 1954. A dehydrated medium for the preparation of type specific extracts of group A streptococci. Appl. Microbiol. **2**:117.
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5. **Facklam, R. R., and R. B. Carey**. 1985. Streptococci and Aerococci, p. 154-175. *In*, E. H.Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
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7. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, p.772-775. vol. 1. Williams & Wilkins, Baltimore, MD.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.

Packaging

Todd Hewitt Broth	100 g	0492-15
	500 g	0492-17
	2 kg	0492-07
	10 kg	0492-08

Tomato Juice Media

Bacto® Tomato Juice Agar · Bacto Tomato Juice Agar Special Bacto Tomato Juice Broth

Intended Use

Bacto Tomato Juice Agar is used for cultivating and enumerating *Lactobacillus* species.

Bacto Tomato Juice Agar Special is used for cultivating and enumerating lactobacilli and other acidophilic microorganisms from saliva and other specimens.

Bacto Tomato Juice Broth is used for cultivating yeasts and other aciduric microorganisms.

Summary and Explanation

In 1925, Mickle and Breed[1] reported the use of tomato juice in culture media used for cultivating lactobacilli. Kulp[2] investigated the use of tomato juice on bacterial development and found that the growth of *L. acidophilus* was enhanced. Tomato Juice Agar, prepared according to Kulp and White's[3] modification, is especially useful in cultivating *L. acidophilus* from clinical specimens and foodstuffs.[4]

Tomato Juice Agar Special is recommended for the direct plate count of lactobacilli from saliva and for cultivation of other acidophilic microorganisms. The number of lactobacilli in saliva is an index of a predisposition to dental caries as described by Jay.[5, 6] Many dentists use the direct count of lactobacilli for the diagnosis of caries.

The acidic pH of Tomato Juice Agar Special encourages growth of lactobacilli while inhibiting growth of accompanying bacteria. This medium is more selective for lactobacilli than Tomato Juice Agar.

Tomato Juice Broth is recommended for use in cultivating and isolating yeasts, lactobacilli and other aciduric microorganisms from clinical specimens and foods.

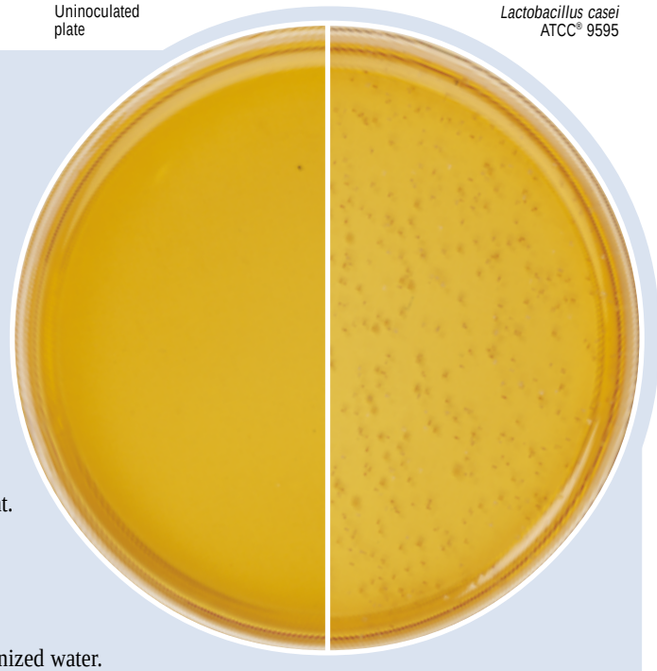
Principles of the Procedure

Tomato Juice Agar and Tomato Juice Agar Special

Tomato Juice is a source of carbon, protein and nutrients. Peptone provides a source of nitrogen, amino acids and carbon. Peptonized Milk contains lactose as an energy source. Bacto Agar is a solidifying agent.

Tomato Juice Broth

Tomato Juice is a source of carbon, protein and nutrients. Yeast Extract is a source of trace elements, vitamins and amino acids. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capability. Magnesium Sulfate, Ferrous Sulfate and Manganese



User Quality Control

Identity Specifications

Tomato Juice Agar

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 5.1% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, very slightly opalescent without precipitate.

Reaction of 5.1% Solution at 25°C: pH 6.1 ± 0.2

Tomato Juice Agar Special

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 6.0% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, slightly opalescent.

Reaction of 6.0% Solution at 25°C: pH 5.0 ± 0.2

Tomato Juice Broth

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 4.1% solution, soluble in distilled or deionized water. Solution is dark amber, clear without significant precipitate.

Reaction of 4.1% Solution at 25°C: pH 6.7 ± 0.2

Cultural Response

Tomato Juice Agar

Prepare Tomato Juice Agar per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus acidophilus	4356	100-1,000	good
Lactobacillus casei	9595	100-1,000	good
Lactobacillus delbrueckii	4797	100-1,000	good

Tomato Juice Agar Special

Prepare Tomato Juice Agar Special per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours (72 hours if necessary).

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus acidophilus	4356	100-1,000	good
Lactobacillus casei	9595	100-1,000	good
Lactobacillus delbrueckii	4797	100-1,000	good

Tomato Juice Broth

Prepare Tomato Juice Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus casei	9595	100-1,000	good
Lactobacillus delbrueckii	4797	100-1,000	good
Saccharomyces carlsbergensis	9080	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

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

Sulfate provide inorganic ions. Sodium Chloride is a source of essential ions that maintain the osmotic balance of the medium.

Formula

Tomato Juice Agar

Formula Per Liter

Tomato Juice (400 ml)	20 g
Bacto Peptone	10 g
Peptonized Milk	10 g
Bacto Agar	11 g

Final pH 6.1 ± 0.2 at 25°C

Tomato Juice Agar Special

Formula Per Liter

Tomato Juice (400 ml)	20 g
Bacto Peptone	10 g
Peptonized Milk	10 g
Bacto Agar	20 g

Final pH 5.0 ± 0.2 at 25°C

Tomato Juice Broth

Formula Per Liter

Tomato Juice (400 ml)	20 g
Bacto Yeast Extract	10 g
Bacto Dextrose	10 g
Dipotassium Phosphate	0.5 g
Monopotassium Phosphate	0.5 g
Magnesium Sulfate	0.2 g
Sodium Chloride	0.01 g
Ferrous Sulfate	0.01 g
Manganese Sulfate	0.01 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. Tomato Juice Agar: For Laboratory Use.
   Tomato Juice Agar Special: For Laboratory Use.
   Tomato Juice Broth: For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

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

Expiration Date

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

Procedure

Materials Provided

Tomato Juice Agar
Tomato Juice Agar Special
Tomato Juice Broth

Materials Required but not Provided

Glassware
Autoclave
Distilled or deionized water

Method of Preparation

Tomato Juice Agar

1. Equilibrate the medium to room temperature before opening.
2. Suspend 51 grams in 1 liter distilled or deionized water.
3. Heat to boiling to dissolve completely.
4. Autoclave at 121°C for 15 minutes.

Tomato Juice Agar Special

1. Equilibrate the medium to room temperature before opening.
2. Suspend 60 grams in 1 liter distilled or deionized water.
3. Heat to boiling to dissolve completely.
4. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.

Tomato Juice Broth

1. Dissolve 41 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Mickle and Breed.** 1925. Technical Bulletin 110. NY State Agriculture Exp. Station.
2. **Kulp, W. L.** 1927. Scientific apparatus and laboratory methods. An agar medium for plating L. acidophilus and L. bulgaricus. Science **66**:512-513.
3. **Kulp, J. W. L., and V. White.** 1932. Modified medium for plating *L. acidophilus*. Science **76**:17-18.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol 1, p. 776-778. Williams & Wilkins, Baltimore, MD.
5. **Jay, P., and S. Gordon (ed).** 1938. Bacteriology and immunology of dental caries and dental science and dental art. Lea and Febiger, Philadelphia, PA.
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Packaging

Tomato Juice Agar	500 g	0031-17
Tomato Juice Agar Special	500 g	0389-17
Tomato Juice Broth	500 g	0517-17
	10 kg	0517-08

Transport Media

Bacto® Transport Medium Amies · Bacto Transport Medium Amies w/o Charcoal · Bacto Transport Medium Stuart · Bacto Cary-Blair Transport Medium

Intended Use

Bacto Transport Medium Amies, Transport Medium Amies w/o Charcoal and Transport Medium Stuart are used for collecting, transporting and preserving microbiological specimens.

Bacto Cary-Blair Transport Medium is used for collecting, transporting and preserving microbiological specimens, particularly those containing *Vibrio cholerae*.

Summary and Explanation

Transport media are chemically defined, semisolid, non-nutritive, phosphate buffered media that provide a reduced environment. Transport media are formulated to maintain the viability of microorganisms without significant increase in growth.

In 1948, Moffett, Young and Stuart described a medium for transporting gonococcal specimens to the laboratory.[1] Stuart, Toshach and Patsula improved this formulation, introducing what is now known as Stuart's Transport Medium.[2] The ability of Stuart's medium to maintain the viability of gonococci during transport[3,4] led other researchers to explore its use with a variety of specimens. This medium is currently recommended for throat, vaginal, and wound samples.

In 1964, Cary and Blair modified Stuart's medium by substituting inorganic phosphates for glycerophosphate and raising the pH to 8.4.[5] The modified medium was effective in maintaining the viability of *Salmonella* and *Shigella*[6,7] in fecal samples. Due to its high pH, Cary-Blair Transport Medium is also effective in maintaining the viability of *Vibrio* cultures for up to four weeks.[8] Cary-Blair Transport Medium is currently recommended for fecal and rectal samples.

Amies[9] confirmed Cary and Blair's observations that an inorganic salt buffer was superior to the glycerophosphate. He further modified the formulation by using a balanced salt solution containing inorganic phosphate buffer, omitting the methylene blue, and adding charcoal. This modified medium yielded a higher percentage of positive cultures than the transport medium of Stuart. Transport Medium Amies, available with and without charcoal, is recommended for throat, vaginal, and wound samples.

User Quality Control

Identity Specifications

Transport Medium Amies

Dehydrated Appearance:	Black, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in distilled or deionized water on boiling. Solution is black, opaque.
Prepared Vials:	Black, opaque, semi-solid.
Reaction of 2.0% Solution at 25°C:	pH 7.3 ± 0.2

Transport Medium Amies w/o Charcoal

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	1.0% solution, soluble in distilled or deionized water on boiling. Solution is colorless to very light amber, opalescent with precipitate.
Prepared Vials:	Colorless to very light amber, opalescent with precipitate, semi-solid.
Reaction of 1.0% Solution at 25°C:	pH 7.3 ± 0.2

Transport Medium Stuart

Dehydrated Appearance:	Bluish white, free-flowing, homogeneous.
Solution:	1.41% solution, soluble in distilled or deionized water on boiling. Solution is light amber, opalescent with a bluish upper layer.
Prepared Vials:	Light amber, opalescent with blue upper layer, without precipitate, semi-solid.
Reaction of 1.41% Solution at 25°C:	pH 7.4 ± 0.1

continued on following page

Principles of the Procedure

In the formulations, potassium chloride, calcium chloride, magnesium chloride and sodium chloride provide essential ions that help maintain osmotic balance while controlling permeability of bacterial cells. Monopotassium phosphate and Disodium phosphate provide buffering capabilities. Sodium thioglycollate suppresses oxidative changes and provides a reduced environment. Sodium glycerophosphate is a buffer for use with calcium chloride. Methylene blue is a colorimetric pH indicator of the oxidation-reduction state. Charcoal neutralizes fatty acids that are toxic to microorganisms. Bacto Agar is a solidifying agent.

Formula

Transport Medium Amies

Formula Per Liter

Sodium Chloride	3 g
Potassium Chloride	0.2 g
Calcium Chloride	0.1 g
Magnesium Chloride	0.1 g
Monopotassium Phosphate	0.2 g
Disodium Phosphate	1.15 g
Sodium Thioglycollate	1 g
Charcoal	10 g
Bacto Agar	4 g

Final pH 7.3 ± 0.2 at 25°C

Transport Medium Amies w/o Charcoal

Formula Per Liter

Sodium Chloride	3 g
Potassium Chloride	0.2 g
Calcium Chloride	0.1 g
Magnesium Chloride	0.1 g
Monopotassium Phosphate	0.2 g
Disodium Phosphate	1.15 g
Sodium Thioglycollate	1 g
Bacto Agar	4 g

Final pH 7.3 ± 0.2 at 25°C

Transport Medium Stuart

Formula Per Liter

Sodium Thioglycollate	0.9 g
Sodium Glycerophosphate	10 g
Calcium Chloride	0.1 g
Bacto Methylene Blue	0.002 g
Bacto Agar	3 g

Final pH 7.4 ± 0.1 at 25°C

Cary-Blair Transport Medium

Formula Per Liter

Sodium Thioglycollate	1.5 g
Disodium Phosphate	1.1 g
Calcium Chloride	0.1 g
Sodium Chloride	5 g
Bacto Agar	5 g

Final pH 8.4 ± 0.2 at 25°C

User Quality Control cont.

Cary-Blair Transport Medium

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	1.27% solution, soluble in distilled or deionized water on boiling. Solution is colorless with a very light amber tint, very slightly to slightly opalescent, may have a slight, fine precipitate.
Prepared Vials:	Colorless to whitish gray, opalescent without significant precipitate, semi-solid.
Reaction of 1.27% Solution at 25°C:	pH 8.4 ± 0.2

Cultural Response

Transport Medium Amies, Transport Medium Amies w/o Charcoal and Transport Medium Stuart

Prepare media per label directions. Inoculate sterile swabs with suspensions of test organisms containing 1,000-10,000 CFU/0.1 ml. Place swabs in the medium and incubate at room temperature for 18-24 hours. Remove swabs, streak on prepared chocolate agar plates and incubate appropriately. All cultures should be viable.

ORGANISM	ATCC®
Bacteroides fragilis	25285*
Haemophilus influenzae Type b	10211
Neisseria meningitidis Group B	13090*
Neisseria gonorrhoeae	43069
Streptococcus pneumoniae	6305
Streptococcus pyogenes Group A	19615*

Cary-Blair Transport Medium

Prepare Cary-Blair Transport Medium per label directions. Inoculate sterile swabs with suspensions of test organisms containing 1,000-10,000 CFU/0.1 ml. Place in the medium, and incubate at room temperature for up to 48 hours. Remove swabs, streak on prepared TSA with 5% Sheep Blood plates and incubate appropriately. All cultures should be viable.

ORGANISM	ATCC®
Salmonella enteritidis	13076
Shigella dysenteriae	13313
Vibrio cholerae biotype *eltor*	15748
Vibrio parahaemolyticus EB 101	–

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. **Transport Medium Amies: 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.

   Transport Medium Amies w/o Charcoal: 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.

   Transport Medium Stuart: 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.

   Cary-Blair Transport 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.

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

Expiration Date

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

Procedure

Materials Provided

Transport Medium Amies, Transport Medium Amies w/o Charcoal, Transport Medium Stuart, or Cary-Blair Transport Medium.

Materials Required but not Provided

Glassware
Autoclave
Distilled or deionized water
Incubator

Method of Preparation

1. **Transport Medium Amies:** Suspend 20 grams in 1 liter distilled or deionized water. Invert vials just before solidification to uniformly distribute the charcoal.

   Transport Medium Amies w/o Charcoal: Suspend 10 grams in 1 liter distilled or deionized water.

   Transport Medium Stuart: Suspend 14.1 grams in 1 liter distilled or deionized water.

   Cary-Blair Transport Medium: Suspend 12.7 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into 6-8 ml capacity screw-cap vials to within 5mm of the top. Cap tightly.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and primary isolation technique recommendations.[10,11,12]

Test Procedure

1. Insert specimen swab(s) into the upper third of the medium in the transport container.
2. Cut or break off the protruding portion of the swab stick. Tightly screw the lid on the bottle or vial.
3. Label the bottle or vial and send to the laboratory with minimum delay. Specimens may be refrigerated until ready for shipment.
4. Submit to laboratory within 24 hours for culture and analysis.

Results

Survival of bacteria in a transport medium depends on many factors including the type and concentration of bacteria in the specimen, the formulation of the transport medium, the temperature and duration of transport, and inoculation to appropriate culture media within 24 hours.

Optimal growth and typical morphology can only be expected following direct inoculation and appropriate cultivation.

Limitations of the Procedure

1. Specimens taken from transport media will not exhibit the optimal or comparative growth as expected from direct inoculation and cultivation. These media do, however, provide an adequate degree of preservation for those specimens which cannot be forwarded immediately to the laboratory for prompt evaluation.
2. Viability of cells will diminish over time and some degree of multiplication or growth of contaminants can occur during prolonged periods of transit. This is particularly true of fecal specimens that contain substantial numbers of coliform organisms.
3. The condition of the specimen received by the laboratory for culture is a significant variable in recovery and final identification of the suspect pathogen. An unsatisfactory specimen (overgrown by contaminants, containing non-viable organisms, or having the number of pathogens greatly diminished) can lead to erroneous or inconclusive results.

References

1. **Moffett, M., J. L. Young, and R. D. Stuart.** 1948. Centralized *gonococcus* culture for dispersed clinics; the value of a new transport medium for gonococci and trichomonas. Brit. Med. J. **2**:421-424.
2. **Stuart, R. D., S. R. Toshach, and T. M. Patsula.** 1954. The problem of transport of specimens for culture of gonococci. Can. J. Public Health **45**:73-83.
3. **Stuart, R. D.** 1946. The diagnosis and control of gonorrhea by bacteriological cultures. Glasgow M. J. **27**:131-143.
4. **Stuart, R. D.** 1959. Transport medium for specimens in public health bacteriology. Public Health Reports **74**:431-438.
5. **Cary, S. G., and E. B. Blair.** 1964. New transport medium for shipment of clinical specimens. J. Bacteriol. **88**:96-98.
6. **Cary, S. G., M. S. Matthew, M. H. Fusillo, and C. Harkins.** 1965. Survival of *Shigella* and *Salmonella* in a new transport medium. Am. J. Clin. Path. **43**:294- 296.
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8. **Kelly, M. T., F. W. Hickman-Brenner, and J. J. Farmer III.** 1991. Vibrio, p. 384- 395. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed.. American Society for Microbiology, Washington D.C.
9. **Amies, C. R.** 1967. A modified formula for the preparation of Stuart's transport medium. Can. J. Public Health **58**:296-300.

10. **Miller, J. M., and H. T. Holmes**. 1995. Specimen collection, transport, and storage, p. 19-31. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
11. **Isenberg, H. D., F. D. Schoenknecht, and A. von Graevenitz.** 1979. Cumitech 9, Collection and processing of bacteriological specimens. Coord. Ed., S. J. Rubin. American Society for Microbiology, Washington, D.C.
12. **Isenberg, H. D. (ed.)**. 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Packaging

Transport Medium Amies	500 g	0996-17
Transport Medium Amies w/o Charcoal	500 g	0832-17
Transport Medium Stuart	500 g	0621-17
Cary-Blair Transport Medium	500 g	0505-17

Trichophyton Agars

Bacto® Trichophyton Agar 1 · Trichophyton Agar 2 · Trichophyton Agar 3 · Trichophyton Agar 4 · Trichophyton Agar 6 Trichophyton Agar 7

Intended Use

Bacto Trichophyton Agars are used for differentiating *Trichophyton* species based on differing nutritional requirements.

Summary and Explanation

Trichophyton Agar media are prepared according to the formulations of Georg and Camp[1] and are based on the nutritional requirements of various species. The growth patterns of the different species are given in Table 1.[1] *(see Table 1 below)*

The authors divided the dermatophytes into four groups. Group 1 includes *T. verucosum*, *T. schoenleinii* and *T. concentricum*. Organisms within this group seldom produce spores or distinctive pigments and their colonies resemble each other so closely that they cannot be identified by morphological criteria. These species are differentiated as indicated in Table 1 by their growth in the absence or presence of inositol, thiamine, or a combination. *T. verrucosum* grows faster at 37°C than at room temperature while *T. schoenleinii* and *T. concentricum* are not stimulated by incubation at 37°C.

Group 2 includes *T. tonsurans*, *T. mentagrophytes* and *T. rubrum*. These species usually produce microconidia, but only occasionally produce macroconidia. Their colonial forms and pigments are so variable that differentiation by these means is inadequate. Ajello and Georg[2] based differentiation on their nutritional behavior on Trichophyton Agars 1 and 4 and by *in vitro* hair cultures. *T. mentagrophytes* forms perforating organs within 2-3 weeks in human hair samples suspended in water; *T. rubrum* does not.

Table 1. Growth patterns of *Trichophyton* species on Trichophyton Agars.[1]

DERMATOPHYTE TESTED	STRAINS STUDIED (1% REPORTING)	TRICHOPHYTON AGARS 1	2	3	4	6	7
T. verrucosum	100 (84%) 100 (16%)	0 0	± 0	4+ 4+	0 4+		
T. schoenleinii	50	4+	4+	4+	4+		
T. concentricum	19 (50%) 19 (50%)	4+ 2+	4+ 2+	4+ 4+	4+ 4+		
T. tonsurans	70	± to 1+			4+		
T. mentagraphytes	50	4+			4+		
T. rubrum	50	4+			4+		
T. ferrugineum	14	4+			4+		
T. violaceum	13	± to 1+			4+		
T. megninii	13					0	4+
T. gallinae	7					4+	4+
*T. equinum**	13	0					

* *T. equinum* will grow to a 4+ reaction on Trichophyton Agar 5, which is not commercially available from Difco Laboratories. Trichophyton Agar 5 is equivalent to Trichophyton Agar 1 (product code 0877) with added nicotinic acid (200 µg per liter).

Group 3 includes *T. violaceum*. It seldom produces microconidia but does develop characteristically pigmented colonies. *T. violaceum* has a similar nutritional pattern as *T. tonsurans*; however, it grows very slowly even in the presence of thiamine and produces a glabrous colony without spores. *T. tonsurans* grows rapidly in the presence of thiamine and shows numerous microconidia.

Group 4 includes *T. megninii* and *T. equinum*. Both can be identified solely from nutritional requirements. *T. megninii* requires histidine, as indicated on Trichophyton Agar 6 in Table 1. *T. equinum* requires nicotinic acid, as indicated in the table.

T. gallinae is differentiated morphologically as well as culturally from *T. megninii* and *T. equinum*. *T. gallinae* grows on Trichophyton Agar 1 or 6 without added vitamins.

Principles of the Procedure

Trichophyton Agars 1, 2, 3 and 4

Vitamin Assay Casamino Acids is the source of nutrients. Dextrose provides carbon. Monopotassium Phosphate provides buffering capability. Magnesium Sulfate is a source of divalent cations and sulfate. Bacto Agar is a solidifying agent. Where included in the formulations for Trichophyton Agars 2,3 and 4, Inositol and Dextrose provide carbon; Thiamin is present for organisms requiring the vitamin for growth.

Trichophyton Agars 6 and 7

Ammonium Nitrate is a source of nitrogen. Dextrose provides carbon. Monopotassium Phosphate provides buffering capability. Magnesium Sulfate is a source of divalent cations and sulfate. Bacto Agar is a solidifying agent. Histidine Hydrochloride is present in Trichophyton Agar 7 for organisms requiring the amino acid histidine.

Formula

Trichophyton 1

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	2.5 g
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Trichophyton Agars 1, 2, 3, 4, 6 and 7

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	5.9% solution, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, slightly opalescent.
Reaction of 5.9% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Trimchophyton Agars 1, 2 and 3

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC®	GROWTH AGARS 1 & 2	GROWTH AGAR 3
Trichophyton concentricum	9358	good	good
Trichophyton schoenleinii	4822	good	good
Trichophyton verrucosum	34470	none to poor	good

Trichophyton Agar 4

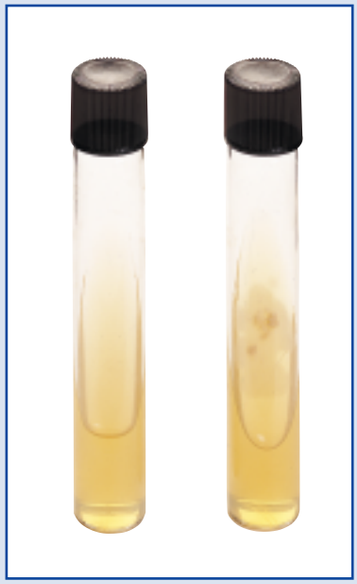
Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC®	GROWTH
Trichophyton rubrum	28188	good
Trichophyton verrucosum	34470	none to poor
Trichophyton violaceum	8376	good

Trichophyton Agar 6

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC®	GROWTH
Microsporum gallinae	22243	good
Trichophyton megninii	12106	none to poor



Uninoculated tube

Trichophyton schoenleinii ATCC® 4822

Trichophyton Agar 7

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC®	GROWTH
Microsporum gallinae	12108	good
Trichophyton megninii	12106	good

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

Trichophyton 2

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	2.5 g
Bacto Inositol	50 mg
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Trichophyton 3

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	2.5 g
Bacto Inositol	50 mg
Thiamin	200 µg
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Trichophyton 4

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	2.5 g
Thiamin	200 µg
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Trichophyton 6

Formula Per Liter

Ammonium Nitrate	1.5 g
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Trichophyton 7

Formula Per Liter

Ammonium Nitrate	1.5 g
Histidine Hydrochloride	30 mg
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

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

Storage

Store dehydrated Trichophyton Agars 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

Trichophyton Agars 1, 2, 3, 4, 6 and 7

Materials Required but not Provided

Glassware
Autoclave

Method of Preparation

1. Suspend 59 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 12 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]

Test Procedure[1,2,4]

1. Inoculate the media by placing a small particle (approximately 1 mm square) of a colony on each medium.
2. Incubate at 30°C for up to 2 weeks.

Results

Examine the media, comparing the amount of growth on each. A small amount of growth is documented as a + and heavy growth as 4+.

Limitations of the Procedure

1. It is important that pure cultures from a medium that is not vitamin enriched, such as Sabouraud Dextrose Agar of Mycobiotic Agar, be used for the inoculum.
2. If cultures are contaminated with bacteria, the cultures should be grown on a medium containing antibiotics, such as Mycobiotic Agar or Brain Heart CC Agar, for several generations to eliminate the bacteria. Many bacteria synthesize vitamins and may invalidate the test results.
3. When inoculating Trichophyton Agars, take care not to carry over growth substances from primary cultures to the tube media used in the differential tests. Inocula transferred to the nutrition tubes should be very small.

References

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Packaging

Trichophyton Agar 1	500 g	0877-17
Trichophyton Agar 2	500 g	0874-17
Trichophyton Agar 3	500 g	0965-17
Trichophyton Agar 4	500 g	0197-17
Trichophyton Agar 6	500 g	0524-17
Trichophyton Agar 7	500 g	0955-17

Bacto® Triple Sugar Iron Agar

Intended Use

Bacto Triple Sugar Iron Agar is used for differentiating gram-negative enteric bacilli based on fermentation of dextrose, lactose and sucrose and on hydrogen sulfide production.

Also Known As

Triple Sugar Iron Agar is also known as TSI.

Summary and Explanation

In 1911, Russell1 described the use of two sugars in an agar medium to differentiate gram-negative organisms of intestinal origin. Lead or iron salts were added to the Russell medium to detect the presence of hydrogen sulfide. Kligler[2,3] added lead acetate to Russell Double Sugar Agar, resulting in a medium that was capable of differentiating typhoid, paratyphoid and dysentery. A modification of this medium, Kligler Iron Agar, using phenol red as an indicator and iron salts to detect hydrogen sulfide production, was developed.

Krumweide and Kohn[4] modified Russell Double Sugar Agar by the addition of sucrose to the medium. This modification allowed for an earlier detection of coliform organisms that ferment lactose slowly, since many of these organisms attack sucrose more readily than lactose. The added sucrose also permitted the exclusion of certain coliform and *Proteus* organisms that can attack sucrose, but not lactose, in a 24-48 hour incubation period.

In 1940, Sulkin and Willet[5] described a triple sugar ferrous sulfate medium for use in the identification of enteric organisms. Difco Laboratories concurrently developed a similar medium by adding 1% sucrose to Kligler Iron Agar with phenol red as the indicator. Hajna[6] described a similar medium for the identification of bacteria of the intestinal group.

Triple Sugar Iron Agar is essentially the formula originally described by Sulkin and Willet.[5] Tryptone has been replaced by a combination of Bacto Peptone and Proteose Peptone. Yeast Extract has been added, and Phenol Red is used as an indicator instead of Brom Thymol Blue.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	6.5% solution, soluble in distilled or deionized water on boiling. Solution is red, very slightly opalescent, may have a slight dark precipitate.
Prepared Tubes:	Red, slightly opalescent, may have a slight precipitate.
Reaction 6.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Triple Sugar Iron Agar per label directions. Inoculate and incubate the tubes at 35°C for 18-24 hours.

ORGANISM	ATCC®	CFU	GROWTH	SLANT /BUTT	GAS	H_2S
Escherichia coli	25922*	undiluted	good	A/A	+	–
Pseudomonas aeruginosa	9027	undiluted	good	K/K	–	–
Salmonella enteritidis	13076	undiluted	good	K/A	+	+
Shigella flexneri	12022*	undiluted	good	K/A	–	–

A = acid reaction (yellow)
K = alkaline reaction (no color change)
+gas = cracks, splits or bubbles in medium
–gas = no cracks, splits or bubbles in medium
+H_2S = black precipitate in butt
–H_2S = no black precipitate in butt

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Salmonella enteritidis* ATCC® 13076 | *Shigella flexneri* ATCC® 12022

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

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

Triple Sugar Iron Agar is recommended for differentiation of enteric gram negative bacilli from clinical specimens,[7,8,9] dairy samples[10], and food samples.[11,12,13,14] Its use is also recommended for microbial limits testing of *Escherichia coli* and *Salmonella* spp.[15]

Principles of the Procedure

Beef Extract, Yeast Extract, Bacto Peptone, and Proteose Peptone provide nitrogen, vitamins, and minerals. Triple Sugar Iron Agar contains three carbohydrates (dextrose, lactose and sucrose). When these carbohydrates are fermented, the resulting production of acid is detected by the phenol red indicator. The color changes that result are yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide. Hydrogen sulfide then reacts with an iron salt yielding the typical black iron sulfide. Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Formula

Triple Sugar Iron Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Yeast Extract	3 g
Bacto Peptone	15 g
Bacto Proteose Peptone	5 g
Bacto Dextrose	1 g
Bacto Lactose	10 g
Sucrose	10 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Bacto Agar	12 g
Bacto Phenol Red	0.024 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

The expiration date applies to the 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

Triple Sugar Iron Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 65 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes with closures.
4. Autoclave at 121°C for 15 minutes. Cool in slanted position with deep butts.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7-14]
2. Process each specimen, using procedures appropriate for that specimen or sample.[7-14]

Test Procedure

1. Obtain a pure culture of the organism to be tested. Select well-isolated colonies.
2. With an inoculating needle, pick the center of well-isolated colonies obtained from solid culture media.
3. Stab the center of the medium into the deep of the tube to within 3 - 5 mm from the bottom.
4. Withdraw the inoculating needle, and streak the surface of the slant.
5. Loosen closure on the tube before incubating.
6. Incubate at 35°C for 18-24 hours.
7. Read tubes for acid production of slant/butt, gas, and hydrogen sulfide reactions.

Results

1. An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only.
2. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose.
3. An alkaline slant-alkaline butt (red/red) indicates that neither dextrose nor lactose was fermented (non-fermenter).
4. Cracks, splits, or bubbles in the medium indicate gas production.
5. A black precipitate in the butt indicates hydrogen sulfide production.

Limitations of the Procedure

1. Hydrogen sulfide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton[15] showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader[16] found that not all H_2S-positive *Salmonella* are positive on TSI.
2. Sucrose is added to TSI to eliminate some sucrose-fermenting non-lactose fermenters such as *Proteus* and *Citrobacter* spp.[17]
3. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.

4. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production.[17]
5. A pure culture is essential when inoculating Triple Sugar Iron Agar. If inoculated with a mixed culture, irregular observations may occur.
6. Tubes should be incubated with caps loosened. This allows a free exchange of air, which is necessary to enhance the alkaline condition on the slant.[17]

References

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Packaging

Triple Sugar Iron Agar	100 g	0265-15
	500 g	0265-17
	2 kg	0265-07

Bacto® Tryptic Nitrate Medium

Intended Use

Bacto Tryptic Nitrate Medium is used for differentiating microorganisms based on nitrate reduction.

Summary and Explanation

Tryptic Nitrate Medium is a differential, semi-solid, general purpose medium that supports growth of aerobes as well as facultative and obligate anaerobes.[1] The formulation includes potassium nitrate which can be reduced by certain organisms to either nitrite or nitrogen gas. Nitrate reduction can be detected by various test methods and is used in differentiating organisms from clinical samples, foods and dairy products.[1,2,3,4,5]

Principles of the Procedure

Tryptose is a source of nitrogen, amino acids, and vitamins. Dextrose provides carbohydrates. Potassium Nitrate provides the basis for nitrate reduction. Disodium Phosphate is a buffering agent. The low agar content, which allows varying degrees of anaerobiosis in the medium, supports growth of organisms with various oxygen requirements.

Formula

Tryptic Nitrate Medium

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	1 g
Disodium Phosphate	2 g
Potassium Nitrate	1 g
Bacto Agar	1 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

Storage

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

Store prepared 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

Tryptic Nitrate Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Test tubes with closures
Autoclave
Incubator (35°C)
SpotTest™ Nitrate Reagents A, B and C or equivalents

Method of Preparation

1. Suspend 25 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes with closures.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

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

Test Procedure

1. Obtain a pure culture of the organism to be tested from a solid culture medium. Select well-isolated colonies.
2. Inoculate a tube of Tryptic Nitrate Medium and incubate at 35 ± 2°C for 18-24 hours.
3. Read tubes for growth.
4. Test for nitrate reduction using SpotTest Nitrate Reagents A, B and C or equivalents per reagent instructions.

Results

1. After adding Nitrate Reagents A and B:
   Positive nitrate reduction reaction: Development of a red-violet color within 1-2 minutes indicates that nitrate has been reduced to nitrite.
   Presumptive negative nitrate reduction reaction: Lack of color development denotes an absence of nitrite in the medium; this should be confirmed by addition of Nitrate Reagent C (zinc dust).
2. After adding Nitrate Reagent C:
   Positive nitrate reduction reaction: Lack of color development indicates that nitrate has been reduced to nitrogen gas.
   Negative nitrate reduction reaction: Development of a red-vio let color within 5- 10 minutes indicates that unreduced nitrate is still present.

Limitations of the Procedure

1. This medium is not recommended for indole testing of coliforms and other enterics.[1]

User Quality Control

Identity Specifications

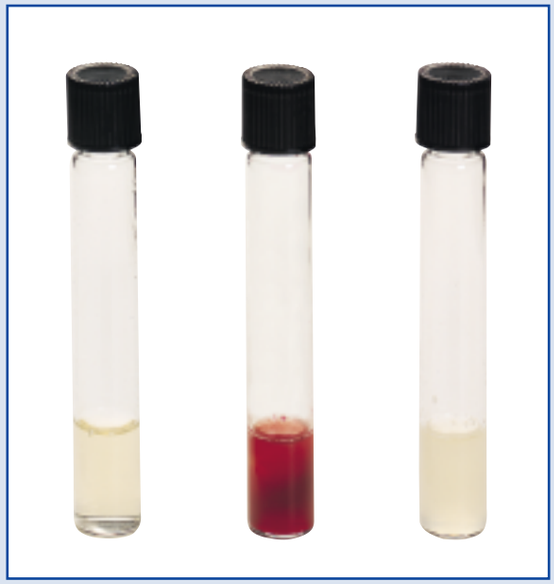
Dehydrated Appearance:	Beige, free flowing, homogeneous.
Solution:	2.5% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent; semisolid.
Reaction of 2.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Tryptic Nitrate Medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours; incubate *Clostridium sporogenes* anaerobically.

ORGANISM	INOCULUM ATCC®	NITRATE CFU	GROWTH	REDUCTION
Clostridium sporogenes	11437	100-1,000	good	–
Escherichia coli	25922*	100-1,000	good	+
Staphylococcus aureus	25923*	100-1,000	good	+

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



Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Clostridium sporogenes* ATCC® 11437

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7. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey and Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Tryptic Nitrate Medium	500 g	0367-17
SpotTest Nitrate Reagent A	50 x 0.75 ml	3554-26
SpotTest Nitrate Reagent B	50 x 0.75 ml	3555-26
SpotTest Nitrate Reagent C	50 x 1 g	3556-26

Bacto® Tryptic Soy Agar

Intended Use

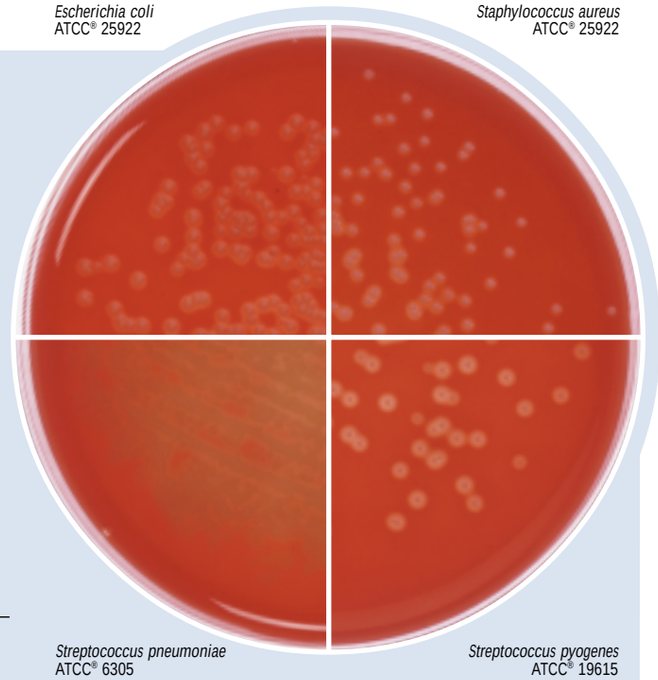
Bacto Tryptic Soy Agar is used for isolating and cultivating fastidious microorganisms and, with blood, in determining hemolytic reactions.

Also Known As

Tryptic Soy Agar (TSA) conforms with Soybean-Casein Digest Agar Medium, USP.

Summary and Explanation

In 1955, Leavitt et al.[1] demonstrated that Tryptic Soy Agar supports excellent growth of a both aerobic and anaerobic microorganisms. Tryptic Soy Agar is a general purpose medium used for multiple applications, e.g., as a blood culture medium, as maintenance medium for culture collections, in colony count methods[2], and for testing bacterial contaminants in cosmetics.[3]



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent.
Plates Prepared Without Blood:	Light amber, slightly opalescent,with no significant precipitate.
Plates Prepared With Blood:	Cherry red, opaque with no hemolysis.
Reaction of 4.0% Solution at 25°C :	pH 7.3 ± 0.2

Cultural Response

Prepare Tryptic Soy Agar per label instructions. Inoculate and incubate the plates at 35 ± 2° under approximately 5-10% 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.

CAMP test: Perform Camp test using *Staphylococcus aureus* ATCC® 33862 or ATCC® 25923. *Streptococcus agalactiae* ATCC® 12386 positive reaction (arrow head area of clearing) *Streptococcus pyogenes* ATCC® 19615 negative reaction (no arrow head formation)

TSA is recommended in multiple water and wastewater applications.[4] Tryptic Soy Agar conforms to the formula specified by US Pharmacopeia for use in Microbiological Tests[5], Antimicrobial Preservatives-Effectiveness and Microbial Limits Test.

Clinically, Tryptic Soy Agar is used in the differentiation of *Haemophilus* species, because it does not contain the X and V factors required for *Haemophilus* growth. With the addition of Differentiation Disks V, X and VX, *Haemophilus* growth can be observed around the appropriate disk.

Tryptic Soy Agar is a nutritious base to which a variety of supplements can be added. Addition of 5% sterile, defibrinated sheep, horse or rabbit blood provides an excellent general purpose medium that allows the growth of yeast species, staphylococci, enterococci and gram-negative bacilli.[6] TSA blood agars may be used to determine hemolytic reactions of bacteria. TSA supplemented with lecithin and polysorbate 80 is the formula for Microbial Content Test Agar (also known as TSALT) used in environmental monitoring.[7] For the examination of foods, Tryptic Soy Agar is supplemented with 3% NaCl for the isolation of *Vibrio* species and halophilic microorganisms.[8] Tryptic Soy Agar supplemented with 0.6% yeast extract is used for the isolation of *Listeria monocytogenes* and cultivation of a wide variety of heterotrophic microorganisms.[8] Addition of colistin and nalidixic acid to TSA is used for the selective isolation of gram-positive cocci.[9] Gunn et al.[10] used trimethoprim and sulfamethoxazole (SxT) supplementation to inhibit normal flora on throat specimens, allowing Groups A and B streptococci to grow well. Addition of iron salt and sodium thiosulfate to TSA aids in the identification of non-fermentating gram-negative bacilli, and with nitro blue tetrazolium (0.5% aqueous solution) allows for the selective isolation of *Corynebacterium diphtheriae*.[11]

Chocolate Agar for culturing *Haemophilus influenzae* and related organisms may be prepared by adding 1% Hemoglobin and Supplement B to Tryptic Soy Agar.

The methodologies for multiple applications using Tryptic Soy Agar are outlined in the references.

Principles of the Procedure

Tryptone and Soytone provide nitrogen, vitamins and minerals. The natural sugars from the soybean promote bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Formula

Tryptic Soy Agar

Formula Per Liter

Bacto Tryptone Pancreatic Digest of Casein	15 g
Bacto Soytone Papaic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Tryptic Soy Agar

Materials Required But Not Provided

Glassware

Autoclave

Incubator (35°C)

Waterbath (45-50°C) (optional)

Defibrinated blood (optional)

Method of Preparation

1. Suspend 40 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

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Packaging

Tryptic Soy Agar	100 g	0369-15
	500 g	0369-17
	2 kg	0369-07
	10 kg	0369-08

Bacto® Tryptic Soy Broth
Bacto Tryptic Soy Broth w/o Dextrose

Intended Use

Bacto Tryptic Soy Broth is used for cultivating a wide variety of microorganisms. Tryptic Soy Broth conforms with the formula specified in the US Pharmacopeia XXIII (USP)[1] and the Code of Federal Regulations (21 CFR)[2] for sterility testing of pharmaceutical products, biologics and devices.

Bacto Tryptic Soy Broth w/o Dextrose, a low carbohydrate formulation of Tryptic Soy Broth, is used for cultivating fastidious and non-fastidious microorganisms.

Also Known As

Tryptic Soy Broth is commonly referred to as Soybean-Casein Digest Medium, USP, and Fluid Soybean-Casein Digest Medium; it is abbreviated as TSB.

User Quality Control

Identity Specifications

Tryptic Soy Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 3.0% Solution at 25°C:	pH 7.3 ± 0.2

Tryptic Soy Broth w/o Dextrose

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.75% solution, soluble in distilled or deionized water; light amber, clear to very slightly opalescent.
Prepared Medium:	Light amber, clear to very slightly opalescent.
Reaction of 2.75% Solution at 25°C:	pH 7.3 ± 0.2

continued on following page

Summary and Explanation

Tryptic Soy Broth is a general purpose medium used for isolating fastidious and non-fastidious microorganisms. Tryptic Soy Broth was originally developed for use without blood in determining the effectiveness of sulfonamides against pneumococci and other organisms.[3] Tryptic Soy Broth is often used to support growth of non-typical isolates such as *Brucella*.[4] Clostridia and non-sporulating anaerobes grow luxuriantly in this broth when incubated under anaerobic conditions. Garrison[5] and Hedgecock[6] used TSB to support growth of *Histoplasma capsulatum*. Mashimo and Ellison[7] supplemented this medium with agar to enhance growth of anaerobic organisms. With the addition of 6.5% NaCl, TSB can be used for the selective growth of group D streptococci.

Tryptic Soy Broth was chosen by the USDA Animal and Plant Health Inspection Service for detecting viable bacteria in live vaccines.[9] It is used in the coliphage detection procedure, a proposed methodology in Standard Methods for the Examination of Water and Wastewater.[10] TSB is recommended for testing bacterial contaminants in cosmetics[11] and complies with established standards[12,13] in the food industry.

TSB is recommended by the National Committee for Clinical Laboratory Standards (NCCLS)[14] for inoculum preparation when performing the disk diffusion sensitivity test, also known as the Kirby-Bauer method.

The rich nutritional base of Tryptic Soy Broth is often modified to provide varying growth environments. With the addition of 1% Supplement B, TSB will support growth of *Neisseria, Haemophilus influenzae* and other related organisms. The medium is used as an enrichment broth in clinical applications and is an excellent blood culture medium when supplemented with SPS and CO_2.[15]

Tryptic Soy Broth w/o Dextrose, a modification of TSB, is a basal medium to which carbohydrates may be added for use in fermentation studies. Agar may be added (0.5-1.0 grams/liter) to enhance anaerobic growth.[16] Phenol red and other indicators may also be added.

Principles of the Procedure

Tryptone and Soytone are nitrogen sources in Tryptic Soy Broth and Tryptic Soy Broth w/o Dextrose. Dextrose is a carbon energy source that facilitates organism growth. Sodium chloride maintains osmotic balance, while dipotassium phosphate is a buffering agent.

Dextrose is omitted from the formula for Tryptic Soy Broth w/o Dextrose to permit use of the medium in fermentation studies. The carbohydrate concentration used most frequently in fermentation reactions is 0.5% or 1%.

Formula

Tryptic Soy Broth

Formula Per Liter

Bacto Tryptone	17 g
Bacto Soytone	3 g
Bacto Dextrose	2.5 g
Sodium Chloride	5 g
Dipotassium Phosphate	2.5 g

Final pH 7.3 ± 0.2 at 25°C

Tryptic Soy Broth w/o Dextrose

Formula Per Liter

Bacto Tryptone	17 g
Bacto Soyton	3 g
Sodium Chloride	5 g
Dipostassium Phosphate	2.5 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

Tryptic Soy Broth
Tryptic Soy Broth w/o Dextrose

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Sterile tubes

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Tryptic Soy Broth - 30 grams;
   Tryptic Soy Broth w/o Dextrose - 27.5 grams.
2. Dispense as desired.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

User Quality Control Cont.

Cultural Response

Prepare Tryptic Soy Broth or Tryptic Soy Broth w/o Dextrose per label directions. Inoculate medium and incubate at 35 ± 2°C for 18-48 hours or up to 76 hours if necessary.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	10-100	fair to good
Staphylococcus epidermidis	12228*	10-100	good
Streptococcus pneumoniae	6305	10-100	good
Streptococcus pyogenes	19615*	10-100	good

USP and EP Growth Promotion[1]

Prepare Tryptic Soy Broth per label directions. Inoculate medium and incubate at the temperature specified for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	INCUBATION TEMPERATURE	RECOVERY
Bacillus subtilis	6633	10-100	20-25°C	growth must be evident
Candida albicans	2091	10-100	20-25°C	growth must be evident
Candida albicans	10231	10-100	20-25°C	growth must be evident
Clostridium sporogenes	19404	10-100	30-35°C	growth must be evident
Staphylococcus aureus	6538P	10-100	30-35°C	growth must be evident

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

Staphylococcus epidermidis ATCC® 12228

Specimen Collection and Preparation

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

Test Procedure

Methodologies for the multiple applications using Tryptic Soy Broth and Tryptic Soy Broth w/o Dextrose are outlined in the references.

Results

Refer to appropriate references and procedures for results.

References

1. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia**,** 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
2. **Federal Register**. 1992. General biological products standards. Fed. Regist. **21**:610.12.
3. **McCullough, N. B.** 1949. Laboratory tests in the diagnosis of brucellosis. Amer. J. of Public Health **39**:866-869.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold**. 1994. Microorganisms encountered in the blood, p. 205. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **Garrison, R. G.** 1961. Studies of the respiratory activity of *Histoplasma capsulatum.* J. of Infect. Dis. **108**:120-124.
6. **Hedgecock, L. W.** 1971. Effect of vaccines prepared from *Histoplasma capsulatum* and other yeast on experimental tuberculosis. J. Bacteria. **82**:115- 123.
7. **Mashimo, P. A., and S. A. Ellison.** 1959. Simple method for the isolation of anaerobic oral vibrios. J. Bacteria. **78**:636-639.
8. **Sherman, J. M., and P. Stark.** 1961. Streptococci which grow at high temperatures. J. Bacteria. **22**:275-285.
9. **Federal Register**. 1992. Detection of viable bacteria and fungi except in live vaccine. Fed. Regist. **21**:113.26.
10. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds).** 1992. Coliphage detection, 9,22-23. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington, D.C.
11. **Curry, A. S., G. G. Joyce, and G. N. McEwen, Jr.** 1993. CTFA Microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Inc., Washington, D.C.
12. **Association of Official Analytical Chemists.** 1995**.** Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
13. **Cunnif, P.** 1995. Official methods of analysis AOAC International, 16th ed. AOAC International, Arlington, VA.
14. **National Committee for Clinical Laboratory Standards.** 1994. Performance standards for antimicrobial disk susceptibility tests, M2-A5, vol. 13, no. 24. National Committee for Clinical Laboratory Standards, Villanova, PA.
15. **Isenberg, H. D. (ed.).** 1992. Processing and interpretation of blood cultures, p. 1.7.1-1.7.2. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
16. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 797, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Tryptic Soy Broth	100 g	0370-15
	500 g	0370-17
	2 kg	0370-07
	10 kg	0370-08
Tryptic Soy Broth w/o Dextrose	500 g	0862-17
	10 kg	0862-08

Bacto® Tryptone

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous powder.
Solution:	1%, 2% and 10% solutions are soluble in distilled or deionized water: 1%-Very light to light amber, clear without precipitate; 2%-Light to medium amber, clear without precipitate; 10%-Medium to dark amber, clear to slightly opalescent, may have a slight precipitate.
Nitrogen (Kjeldahl Method):	11.4-13.9%
Amino Nitrogen (Modified Sorensen Method):	4.0-6.6%
Reaction of 2% Solution at 25°C:	pH 6.9-7.4

continued on following page

Intended Use

Bacto Tryptone is used in preparing microbiological culture media.

Also Known As

Tryptone is also referred to as Peptone C, Peptone 50 and Tryptone T.

Summary and Explanation

Tryptone is a pancreatic digest of casein used as a nitrogen source in culture media formulated for isolating and cultivating fastidious and nonfastidious bacteria and fungi.

Tryptone was developed by Difco Laboratories while investigating a peptone particularly suitable for the elaboration of indole by bacteria. The high tryptophane content of Tryptone makes it valuable for use in detecting indole production.[1,2,3] The absence of detectable levels of carbohydrates in Tryptone makes it a suitable peptone in differentiating bacteria on the basis of their ability to ferment various carbohydrates.

Several media containing Tryptone are specified in standard methods[4,5,6,7] for multiple applications.

Principles of the Procedure

Tryptone is a pancreatic digest of casein especially rich in tryptophane. Casein, a milk protein, is a rich source of amino acid nitrogen.

Typical Analysis

Physical Characteristics

Ash (%)	6.8	Loss on Drying (%)	3.7
Clarity, 1% Solution (NTU)	0.5	pH, 1% Solution	7.2
Filterability (g/cm^2)	1.3		

Carbohydrate (%)

Total	7.7

Nitrogen Content (%)

Total Nitrogen	13.0	AN/TN 20.7	40.0
Amino Nitrogen	5.2		

Amino Acids (%)

Alanine	2.86	Lysine	6.70
Arginine	3.03	Methionine	2.57
Aspartic Acid	6.11	Phenylalanine	3.71
Cystine	0.42	Proline	7.45
Glutamic Acid	17.05	Serine	4.29
Glycine	1.75	Threonine	3.58
Histidine	2.02	Tryptophan	0.71
Isoleucine	4.40	Tyrosine	1.42
Leucine	7.11	Valine	5.00

Inorganics (%)

Calcium	0.013	Phosphate	2.669
Chloride	0.186	Potassium	0.229
Cobalt	<0.001	Sodium	2.631
Copper	<0.001	Sulfate	0.241
Iron	<0.001	Sulfur	0.740
Lead	<0.001	Tin	<0.001
Magnesium	0.017	Zinc	0.003
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.1	PABA	3.7
Choline (as Choline Chloride)	350.0	Pantothenic Acid	5.3
Cyanocobalamin	<0.1	Pyridoxine	0.6
Folic Acid	0.3	Riboflavin	<0.1
Inositol	1400.0	Thiamine	0.4
Nicotinic Acid	97.8	Thymidine	93.4

Biological Testing (CFU/g)

Coliform	negative
Salmonella	negative
Spore Count	73
Standard Plate Count	870
Thermophile Count	8

The values presented are "typical". This information is for broad comparison use only and is not indicative of the makeup of any particular lot of material. No guarantee is made, either expressed or implied, that any specific lot of product will match the values presented.

Procedure

Materials Provided

Tryptone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Tryptone in the formula of the medium being prepared. Add Tryptone 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 Tryptone.

Results

See appropriate references and procedures for results.

References

1. J. Bacteriol. 1933. **25**:623.
2. Pure Culture Study of Bacteria. 1947. No. 3
3. Centr. Bakt. , I Abt. 1915. **76**:1.

User Quality Control cont.

Cultural Response

For each Test specified, prepare a Test Solution of Tryptone and, if necessary, adjust to pH 7.2-7.4; sterilize, inoculate and incubate according to standard test procedure.

TEST	TEST SOLUTION	ORGANISM	ATCC®	INOCULUM	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	1 drop, undiluted	negative; red color
Indole Production	0.1%	*Escherichia coli*	25922*	1 drop, undiluted	positive; pink color on Indole Test Strip
Acetylmethylcarbinol Production	0.1% w/ 0.5% dextrose	*Enterobacter aerogenes*	13048*	1 drop, undiluted	positive; pink color upon adding reagents
Hydrogen Sulfide Production	1%	*Salmonella typhi*	6539	1 drop, undiluted	positive; brownish blackening of H_2S Test Strip
Growth Production	2% w/1.5% agar and 0.5% NaCl	*Escherichia coli*	25922*	100-1,000 CFU	good growth
Growth Production	2% w/1.5% agar and 0.5% NaCl	*Staphylococcus aureus*	25923*	100-1,000 CFU	good growth

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

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

4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual , 8th ed. AOAC International, Gaithersburg, MD.
6. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
7. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Tryptone	100 g	0123-15
	500 g	0123-17
	2 kg	0123-07
	10 kg	0123-08

Bacto® Tryptone Glucose Extract Agar
Bacto m TGE Broth

Intended Use

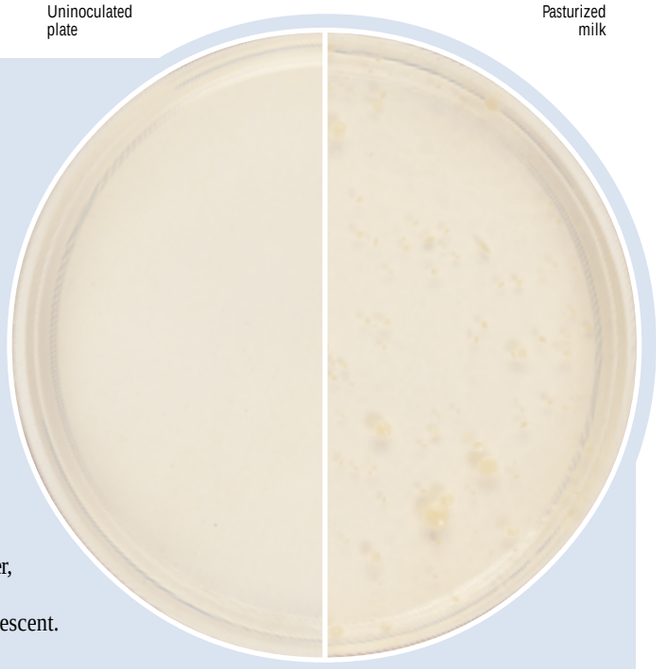
Bacto Tryptone Glucose Extract Agar is used for cultivating and enumerating microorganisms in water and dairy products.

Bacto m TGE Broth is used for enumerating microorganisms by membrane filtration.

Also Known As

Tryptone Glucose Extract Agar is also known as Yeast Dextrose Agar and may be abbreviated as TGEA.

m TGE is an abbreviation for membrane Tryptone Glucose Extract.



User Quality Control

Identity Specifications

Tryptone Glucose Extract Agar

Dehydrated Appearance:	Light to medium tan, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear to slightly opalescent, no significant precipitate.
Prepared Medium:	Light amber, clear to slightly opalescent, no precipitate.
Reaction of 2.4% Solution at 25°C:	pH 7.0 ± 0.2

m TGE Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	1.8% solution, soluble in distilled or deionized water. Solution is medium amber, clear to very slightly opalescent.
Prepared Medium:	Medium amber, clear to very slightly opalescent.
Reaction of 1.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Tryptone Glucose Extract Agar

Prepare Tryptone Glucose Extract Agar per label directions in parallel with a reference control. Inoculate with pasteurized and raw milk samples using the pour plate technique and incubate at 32 ± 1°C for 47-49 hours. Recovery of bacteria from the milk samples should be comparable for both the test and reference lots.

m TGE Broth

Prepare mTGE Broth per label directions. Inoculate using the membrane filter technique and incubate in a humid atmosphere at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good

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

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

Summary and Explanation

In the 1930's, Bower and Hucker[1] developed a medium for detecting bacteria in milk and other dairy products. Many investigators compared the performance of Tryptone Glucose Skim Milk Agar to Nutrient Agar for estimating bacteria in milk and other dairy products.[2,3,4] Prickett[5] used a glucose agar containing Tryptone to study thermophilic bacteria in milk. This medium, described in *Standard Methods of Milk Analysis*[6], was prepared in the dehydrated form as Yeast Dextrose Agar. The American Public Health Association (APHA) adopted Tryptone Glucose Extract Agar for use in testing milk and dairy products in 1948.[7] For many years, Tryptone Glucose Extract Agar with added milk remained the Standard Methods medium for dairy products[8] and was also adopted for testing water.[9] Currently, the APHA specifies using Tryptone Glucose Extract Agar for the heterotrophic plate count procedure in testing bottled water.[10]

m TGE Broth is a nonselective nutrient medium for the determination of bacterial counts by the membrane filter method. The broth has the same formulation as Tryptone Glucose Extract Agar, except that the broth contains no agar and the ingredients are at twice the concentration.

Principles of the Procedure

Tryptone Glucose Extract Agar and m TGE Broth contain Beef Extract and Tryptone as sources of carbon, nitrogen, vitamins and minerals. Dextrose (Glucose) is a carbohydrate. Tryptone Glucose Extract Agar contains Bacto Agar as a solidifying agent.

Formula

Tryptone Glucose Extract Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Tryptone	5 g
Bacto Dextrose (Glucose)	1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

m TGE Broth

Formula Per Liter

Bacto Beef Extract	6 g
Bacto Tryptone	10 g
Bacto Dextrose	2 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Tryptone Glucose Extract Agar
m TGE Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Petri dishes (Tryptone Glucose Extract Agar)
Sterile membranes (m TGE Broth)
Filter apparatus (m TGE Broth)
Sterile absorbent pads (m TGE Broth)
Incubator (TGEA - 32 ± 1°C; mTGE Broth - 35°C)

Method of Preparation

Tryptone Glucose Extract Agar (TGEA)

1. Suspend 24 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

m TGE Broth

1. Dissolve 18 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Collect samples in accordance with laboratory procedures.[10]

Test Procedure

1. Use the appropriate culture method, as follows:
   TGEA - pour plate method;
   m TGE - membrane filtration.
2. Incubate the inoculated medium in a humid atmosphere, as follows:
   TGEA - 32 ± 1°C for 47-49 hours;
   m TGE - 35 ± 2°C for 18-24 hours.

Results

Count total colonies and record 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. **Bowers and Hucker**. 1935. Tech. Bull. 228. NY State Agr. Exp. Sta.
2. **Yale**. 1938. Am. J. Pub. Health **28**:148.
3. **Proc. 36th Cong. Intern. Assoc. Ice Cream Manufacturers**. 1936. **2**:132.
4. **Dennis and Weiser**. 1937. J. Dairy Science **20**:445.
5. **Prickett**. 1928. Tech. Bull. 147. NY State Agr. Exp. Sta.
6. **Standard Methods of Milk Analysis, 6th ed.** 1934.
7. **American Public Health Association**. 1948. Standard methods for the examination of dairy products, 9th ed. American Public Health Association, Washington, D.C.
8. **American Public Health Association**. 1972. Standard methods for the examination of dairy products, 13th ed. American Public Health Association, Washington, D.C.

9. **American Public Health Association**. 1980. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington, D.C.
10. **Cowman, S., and R. Kelsey.** 1992. Bottled water, p. 1031-1036. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Tryptone Glucose Extract Agar	100 g	0002-15
	500 g	0002-17
	2 kg	0002-07
m TGE Broth		
	100 g	0750-15
	500 g	0750-17

Bacto® Tryptone Water

Intended Use

Bacto Tryptone Water is recommended for use in the detection of *Escherichia coli* in food and water samples based on indole production.

Summary and Explanation

Tryptone Water is based on the Tryptone Water formula described in AFNOR and ISO Standards.[1] In these procedures, Tryptone Water is used with Brilliant Green Bile 2% to determine the most probable number (MPN) of *E. coli* present in meat and meat products. Growth and gas production in Brilliant Green Bile 2% and indole production in Tryptone Water following incubation of both media at 44 ± 1°C is used as the basis for this presumptive *E. coli* test.

Tryptone Water may also be used for differentiation of bacteria based on indole production. Production of indole using pure cultures in tryptophan containing media is recommended as an aid in the differentiation of bacteria and the identification of *E. coli* isolated from food and water samples.[2,3]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	1.5 % solution, soluble in distilled or deionized water. Solution is light to medium amber, clear to slightly opalescent with no significant precipitate.
Prepared Medium:	Light to medium amber, clear to slightly opalescent with no significant precipitate.
Reaction of 1.5% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Tryptone Water per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-24 hours. Add 0.5 ml SpotTest™ Indole Reagent Kovacs to the tubes to test for indole production. Formation of a red color denotes a positive indole test.

ORGANISM	ATCC®	INOCULUM CFU	INDOLE PRODUCTION	GROWTH
Escherichia coli	25922*	100-300	positive	good
Enterobacter cloacae	13047	100-300	negative	good

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

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

Principles of the Procedure

Tryptone Water is similar to Tryptone Broth, containing both Tryptone (1%) and Sodium Chloride. Due to its high tryptophan content, Tryptone is suitable for use in detecting indole production by bacteria. Tryptophan is hydrolyzed and deaminated to produce indole, pyruvic acid and ammonia.[4] Indole can then be detected by the addition of either Kovac's or Ehrlich's Reagent, which contain an aldehyde group. The aldehyde group combines with indole to produce a red color in the alcohol layer. Sodium Chloride is added to the medium to provide a suitable osmotic environment.

Formula

Tryptone Water

Formula Per Liter

Bacto Tryptone	10 g
Sodium Chloride	5 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 powder is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Tryptone Water

Materials Required But Not Provided

Brilliant Green Bile 2%
SpotTest™ Indole Reagent Kovacs
Flasks with closures
Distilled or deionized water
Autoclave
Incubator (30 ± 1°C)
Incubator (44 ± 1°C)
Diluent

Method of Preparation

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

Specimen Collection and Preparation

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

Test Procedure

Presumptive Test For *E. coli* in Meats and Meat Products[1]

1. Suspend one part sample in 9 parts diluent. Homogenize sample.
2. Dilute homogenate in duplicate using serial 10-fold dilutions to 10^{-6} using 1 ml of material to be diluted to 9 ml of diluent. Mix each dilution thoroughly.
3. Transfer 1 ml of homogenate (step 1) to 6 tubes containing 10 ml Brilliant Green Bile 2% and group in 2 sets of 3 tubes each. If the number of coliforms is expected to be high, transfer 10 ml of homogenate into 6 tubes containing double strength Brilliant Green Bile 2%.
4. Transfer 1 ml from each of the dilutions prepared in step 2 into each of 3 tubes containing 10 ml Brilliant Green Bile 2%.
5. Incubate Brilliant Green Bile 2% tubes (prepared in steps 3 and 4) at 30 ± 1°C for 48 ± 2 hours. Subculture all tubes containing gas, using 1 drop of inoculum, into Tryptone Water and Brilliant Green Bile 2%.
6. Incubate tubes prepared in step 5 at 44 ± 1°C for 48 ± 2 hours.

Indole Determination Using Pure Cultures

1. Inoculate Tryptone Water using a light inoculum of an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C with loosened caps for 18-24 hours.
3. Add 0.5 ml of SpotTest™ Indole Reagent Kovacs directly to the tube and agitate. Allow tubes to stand for 5-10 minutes.

Results

Presumptive Test For *E. coli* in Meats and Meat Products

Examine and record the tubes of Brilliant Green Bile 2% tubes containing gas. Add 0.5 ml SpotTest™ Indole Reagent Kovacs to the Tryptone Water tubes. Observe tubes for the formation of a red ring at the top of medium indicating indole production. Record the tubes for positive indole production. Determine the MPN (Most Probable Number) of *E. coli* present in the sample based on the number of tubes that are positive for both gas and indole. Consult the appropriate 3-tube MPN table.[2]

Indole Determination Using Pure Cultures

Examine tubes for the formation of a red ring at the top of the tube indicating indole production.

Limitations of the Procedure

1. Detection of *E. coli* in meats using Tryptone Water is a presumptive test. If confirmatory testing is required, please consult appropriate references.
2. Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production. For complete identification of the organism, further biochemical evaluation is necessary.

References

1. **International Organization for Standardization:** Meat and meat products. Detection and enumeration of presumptive coliform bacterial and presumptive *E. coli* (Reference method ISO/DIS 3811-1979).
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. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (ed.).** 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **MacFaddin, J. F.** Biochemical Test for Identification of Medical Bacteria, 3rd ed. 1985. Williams and Wilkens, Baltimore, MD.

Packaging

Tryptone Water	500 g	0644-17

Bacto® Tryptose

Intended Use

Bacto Tryptose is an enzymatic digest of protein for use in preparing microbiological culture media.

Also Known As

Tryptose is also referred to as Polypeptone™ Peptone.

Summary and Explanation

Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. Tryptose was originally developed as a peptone particularly adapted to the growth requirements of *Brucella*. An agar medium containing Tryptose, sodium chloride and dextrose, without liver or other infusions, was shown to be an excellent medium for propagation of these organisms.[1] Culture media prepared with Tryptose were superior to the meat infusion peptone media previously used for the cultivation of *Brucella*, streptococci, pneumococci, meningococci and other fastidious bacteria.[2,3]

An agar medium prepared with 2% Tryptose and 0.5-0.8% sodium chloride, without tissue infusion, is an excellent blood agar base. The growth of organisms on Tryptose Blood Agar Base is luxuriant and the zones of hemolysis produced are distinct and clear. Huddleson[1] used a broth containing 2% Tryptose as an enrichment medium in the isolation of *Brucella* from clinical specimens.

Principles of the Procedure

Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process in Tryptose results in assorted peptides, including those of higher molecular weight.

Typical Analysis

Physical Characteristics

Ash (%)	9.7	Loss on Drying (%)	3.2
Clarity, 1% Soln (NTU)	0.8	pH, 1% Soln	7.4
Filterability (g/cm^2)	2.3		

Carbohydrate (%)

Total	7.1

Nitrogen Content (%)

Total Nitrogen	13.4	AN/TN	32.5
Amino Nitrogen	4.4		

Amino Acids (%)

Alanine	4.45	Lysine	4.64
Arginine	4.65	Methionine	1.92
Aspartic Acid	6.34	Phenylalanine	7.52
Cystine	0.44	Proline	6.33
Glutamic Acid	13.92	Serine	4.09
Glycine	2.84	Threonine	3.55
Histidine	<0.01	Tryptophan	0.62
Isoleucine	0.34	Tyrosine	2.21
Leucine	3.67	Valine	1.93

Inorganics (%)

Calcium	0.001	Phosphate	2.144
Chloride	0.886	Potassium	0.679
Cobalt	<0.001	Sodium	3.410
Copper	<0.001	Sulfate	0.308
Iron	0.002	Sulfur	0.737
Lead	<0.001	Tin	<0.001
Magnesium	0.022	Zinc	0.005
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	11.4
Choline (as Choline Chloride)	2700.0	Pantothenic Acid	16.0
Cyanocobalamin	<0.1	Pyridoxine	1.4
Folic Acid	0.4	Riboflavin	4.3
Inositol	5400.0	Thiamine	0.1
Nicotinic Acid	47.4	Thymidine	769.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	825
Salmonella	negative	Thermophile Count	100
Spore Count	875		

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.
2% - Medium amber, clear to slightly opalescent.
10% - Medium to dark amber, very slightly opalescent to opalescent, may have precipitation.

Reaction of 1% Solution at 25°C: pH 7.1 to 7.5

Cultural Response

TEST	SOLUTION	ORGANISM	ATCC®	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	negative
Indole Production	0.1%	*Escherichia coli*	25922*	positive
Acetylmethyl-carbinol	0.1% w /0.5% dextrose	*Enterobacter aerogenes*	13048*	positive
Hydrogen Sulfide Production	1%	*Salmonella typhi*	6539	positive
Growth Response	2% w/ 0.5% NaCl, 0.1% agar, and 0.1% dextrose	*Brucella suis*	4314	good growth
Growth Response	2% w/ 0.5% NaCl, 0.1% agar, and 0.1% dextrose	*Staphylococcus aureus*	25923*	good growth
Growth Response	2% w/ 0.5% NaCl, 0.1% agar, and 0.1% dextrose	*Streptococcus pneumoniae*	6303*	good growth
Growth Response	2% w/ 0.5% NaCl, 0.1% agar, and 0.1% dextrose	*Streptococcus pyogenes*	19615*	good growth

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

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

Precautions

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

Storage

Store the dehydrated ingredient below 30°C. The 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

Tryptose

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Tryptose in the formula of the medium being prepared. Add Tryptose as required.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Huddleson, I. F.** 1939. Brucellosis in man and animals. **14**. Oxford University Press, Oxford.

2. **Casman, E. P.** 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. J. Bacteriol. **43**:33.
3. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.

Packaging

Tryptose	100 g	0124-15
	500 g	0124-17
	2 kg	0124-07
	10 kg	0124-08

Bacto® Tryptose Agar
Bacto Tryptose Broth

Intended Use

Bacto Tryptose Agar is used for cultivating a wide variety of fastidious microorganisms, particularly for isolating *Brucella* according to Huddleson and Castañeda.

Bacto Tryptose Broth is used for cultivating *Brucella* and other fastidious microorganisms.

User Quality Control

Identity Specifications

Tryptose Agar

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	4.1% solution, soluble on boiling in distilled or deionized water; light amber, very slightly to slightly opalescent, without significant precipitate.
Prepared Medium:	Light amber, slightly opalescent, without precipitate.
Reaction of 4.1% Solution at 25°C:	pH 7.2 ± 0.2

Tryptose Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.6% solution, soluble in distilled or deionized water; light amber, clear, without significant precipitate.
Prepared Medium:	Light amber, clear without significant precipitate.
Reaction of 2.6% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Tryptose Agar and Tryptose Broth per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella abortus	4315	100-1,000	good
Brucella melitensis	4309	100-1,000	good
Brucella suis	6597	100-1,000	good

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

Summary and Explanation

Tryptose media, prepared without extract or infusion of meat, are recommended for the cultivation and isolation of pathogenic and saprophytic bacteria. Historically, it was considered necessary to include meat extract or infusion as a nutritional supplement in culture media. Tryptose was developed while studying the growth requirements of *Brucella*. Huddleson[1] found tryptose media to be equal or superior to meat infusion media, providing uniformity for the cultivation and differentiation of fastidious organisms.

Tryptose media are particularly well suited for the isolation of *Brucella* from blood. Castañeda[2] studied the isolation of *Brucella* species using a broth containing 2% Tryptose and 2% sodium citrate. Sodium citrate serves as an anticoagulant and assists in inactivating complement in the blood specimen.

Tryptose Broth can be used as a complete basal medium or supplemented with enrichments. Huddleson[3] used a broth containing 2% Tryptose as an enrichment medium in the isolation of *Brucella* from clinical specimens. McCullough *et al*. reported that addition of thiamine, dextrose and iron salts increased growth of *Brucella suis*.[4] Addition of 0.1% agar to Tryptose Broth can increase growth of aerobes and anaerobes in liquid media. Blood agar may be prepared by adding 5% sterile, defibrinated sheep, horse or rabbit blood to the sterile medium.

The high productivity of tryptose media in the isolation and cultivation of *Brucella* supports use of these formulas as general purpose media, especially when avoidance of animal tissue products is desired. Tryptose Agar with 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae.[5] For isolation of *Brucella* stains from contaminated milk, crystal violet (gentian violet) can be added to Tryptose Agar to suppress gram-positive organisms.[6] Tryptose media can be supplemented with thiamine or citrate for the cultivation and maintenance of fastidious aerobic and facultative microorganisms.[7]

Tryptose Agar is specified in the Compendium of Methods for the Microbiological Examination of Food.[8] Tryptose media are recommended in FDA Bacteriological Analytical Manual for serological testing.[9]

Principles of the Procedure

In Tryptose media, Tryptose is a source of nitrogen and carbon. Dextrose is a source of carbohydrate. Sodium Chloride maintains osmotic balance. Bacto Agar is the solidifying agent in Tryptose Agar.

Formula

Tryptose Agar

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	1 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Tryptose Broth

Formula Per Liter

Bacto Tryptose	20 g
Sodium Chloride	5 g
Bacto Dextrose	1 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

Storage

Store the dehydrated 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

Tryptose Agar
Tryptose Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes and/or tubes
Bacto Agar (optional)
Sterile defibrinated blood

Method of Preparation

Tryptose Agar

1. Suspend 41 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Distribute into tubes, bottles or flasks.
4. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
5. To prepare blood agar, aseptically add 5% sterile defibrinated sheep, horse or rabbit blood. Dispense into sterile Petri dishes.

Tryptose Broth

1. Dissolve 26 grams in 1 liter distilled or deionized water.
2. Distribute into tubes, bottles or flasks.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.

Specimen Collection and Preparation

Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

Methodologies for the multiple applications using tryptose media are outlined in the references.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Tryptose media are general purpose, non-selective media. Although certain diagnostic tests may be performed directly on the medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification.
2. When preparing blood agar, hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood.
3. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[10] For optimal performance, incubate tryptose media supplemented with blood under increased CO_2 or anaerobic conditions.
4. Dextrose has been shown to inhibit hemolysin production by some organisms.

References

1. **Huddleson, I. F.** 1943. Brucellosis in man and animals. Rev. Ed. The Commonwealth Fund, New York.
2. **Castañeda, M. R.** 1947. A practical method for routine blood cultures in brucellosis. Proc. Soc. Exp. Biol. Med. **64**:114-115.
3. **Huddleson, I. F.** 1939. Brucellosis in man and animals. 14. Oxford University Press, Oxford.
4. **McCullough, W. G., R. C. Mills, E. J. Herbst, W. G. Roessler, and C. R. Brewer.** 1947. Studies on the nutritional requirements of *Brucella suis*. J. Bacteriol. **53**:5-15.
5. **Moyer, N. P., and L. A. Holcomb.** 1995. Brucella. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 802-806. Williams & Wilkins, Baltimore, MD.
7. **Atlas, R. M.** 1995. Handbook of microbiology media for the examination of food, p. 266-268. CRC Press, Boca Raton, FL.
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. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
10. **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

Tryptose Agar	500 g	0064-17
	2 kg	0064-07
	10 kg	0064-08
Tryptose Broth	500 g	0062-17
	10 kg	0062-08

Bacto® Tryptose Blood Agar Base
Bacto Tryptose Blood Agar Base w/Yeast Extract

Intended Use

Bacto Tryptose Blood Agar Base is used with blood in isolating, cultivating and determining the hemolytic reactions of fastidious microorganisms.

Bacto Tryptose Blood Agar Base w/Yeast Extract is used with or without blood in cultivating a wide variety of microorganisms.

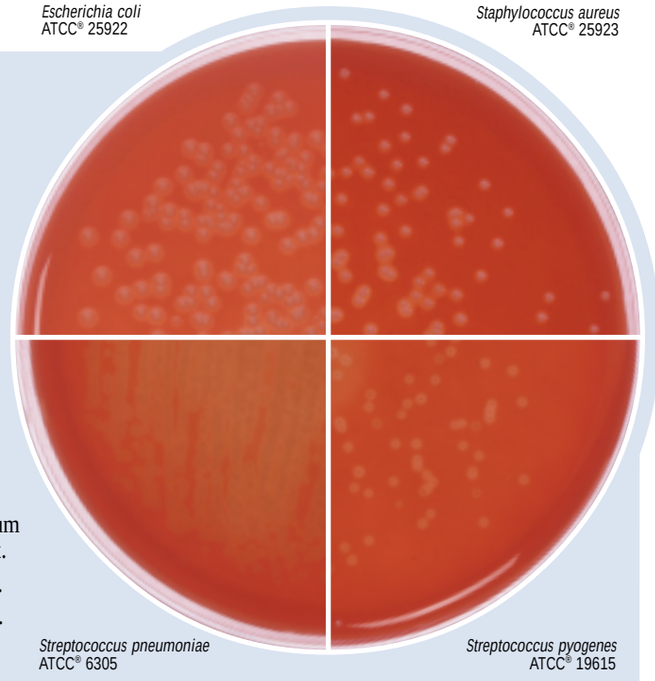
Also Known As

"Blood Agar Base" may be abbreviated as BAB.

Summary and Explanation

Investigations of the nutritive properties of Tryptose demonstrated that culture media prepared with this peptone were superior to the meat infusion peptone media previously used for the cultivation of *Brucella*, streptococci, pneumococci, meningococci and other fastidious bacteria. Casman[1,2] reported that a medium consisting of 2% Tryptose, 0.3% Beef Extract, 0.5% NaCl, 1.5% Bacto Agar and 0.03% dextrose equaled fresh beef infusion base with respect to growth of organisms. The small amount of carbohydrate was noted to interfere with hemolytic reactions, unless the medium was incubated in an atmosphere of carbon dioxide.

Tryptose Blood Agar Base and Tryptose Blood Agar Base w/ Yeast Extract are nutritious infusion-free basal media 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, these bases can be used as general purpose media. Tryptose Blood Agar Base is specified in FDA Bacteriological Analytical Manual.[4] Tryptose Blood Agar Base w/ Yeast Extract was formulated to provide a base with additional nutrients to improve the growth of fastidious organisms.



User Quality Control

Identity Specifications

Tryptose Blood Agar Base

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 3.3% solution, soluble in distilled or deionized water on boiling; light amber, slightly opalescent.

Prepared Medium: Light amber, slightly opalescent. With 5% sheep blood-cherry red, opaque.

Reaction of 3.3% Solution at 25°C: pH 7.2 ± 0.2

Tryptose Blood Agar Base w/Yeast Extract

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 3.4% 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. With 5% sheep blood-cherry red, opaque.

Reaction of 3.4% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Prepare Tryptose Blood Agar Base and Tryptose Blood Agar Base w/ Yeast Extract 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 w/o BLOOD	GROWTH W/BLOOD	HEMOLYSIS 18-48 H
Escherichia coli	25922*	100-1,000	good to excellent	good to excellent	beta
Neisseria meningitidis	13090*	100-1,000	fair to good	good	N/A
Staphylococcus aureus	25923*	100-1,000	good	good	beta
Streptococcus pneumoniae	6305	100-1,000	fair to good	good	alpha
Streptococcus pyogenes	19615*	100-1,000	fair to 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.

Principles of the Procedure

Tryptose is the source of nitrogen, carbon and amino acids in Tryptose Blood Agar Base and Tryptose BAB w/ Yeast Extract. Beef Extract provides additional nitrogen. Bacto Agar is the solidifying agent. Sodium Chloride maintains osmotic balance. Yeast Extract supplies additional vitamins and cofactors for growth.

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

Formula

Tryptose Blood Agar Base

Formula Per Liter

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

Final pH 7.2 ± 0.2 at 25°C

Tryptose Blood Agar Base w/Yeast Extract

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Bacto Yeast Extract	1 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.1 at 25°C

Precautions

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

Storage

Store 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

Tryptose Blood Agar Base

Tryptose Blood Agar Base w/ Yeast Extract

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes
5% sterile defibrinated blood (optional)

Method of Preparation

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

Tryptose Blood Agar Base	33 g
Tryptose Blood Agar Base w/ Yeast Extract	34 g

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

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[7] Obtain and process specimens according to the procedures established by institutional policy. Specimens should be collected in sterile containers or with sterile swabs, and transported 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 of both oxygen-stable and oxygen-labile streptolysins.[3]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.
3. Examine plates for growth and hemolytic reactions after 18-24 and 48-hour incubation. Four different types of hemolysis on blood agar media can be described:[5]
   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 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.[3]

4. 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*.[6]
5. The atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[3] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.
6. Hemolytic patterns may vary with the source of animal blood or type of base medium used.[3]

References

1. **Casman, E. P.** 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. J. Bacteriol. **43**:33.
2. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
3. **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.
4. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel**. 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
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

Tryptose Blood Agar Base	500 g	0232-17
	2 kg	0232-07
Tryptose Blood Agar Base w/ Yeast Extract	500 g	0662-17

Bacto® Tryptose Phosphate Broth

Intended Use

Bacto Tryptose Phosphate Broth is used for cultivating fastidious microorganisms.

Also Known As

Tryptose Phosphate Broth is abbreviated as TPB.

Summary and Explanation

Tryptose Phosphate Broth is an infusion-free buffered medium recommended for the cultivation of fastidious, pathogenic microorganisms. In a study by Waisbren, Carr, and Dunnett,[1] Tryptose Phosphate Broth was used in the tube method for antibiotic sensitivity testing.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.95% solution, soluble in distilled or deionized water; light amber, may be very slightly opalescent with a very slight precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent, may have a very slight precipitate.
Reaction of 2.95% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Tryptose Phosphate Broth 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
Staphylococcus epidermidis	12228*	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.

Uninoculated tube

Streptococcus pneumoniae ATCC® 6305

Tryptose Phosphate Broth is valuable in tissue culture procedures, as shown by Ginsberg, Gold and Jordan.[2] The proteose content of Tryptose Phosphate Broth is considered to be a stimulating factor for cells. Tryptose Phosphate Broth is specified in the FDA Bacteriological Analytical Manual for cell culture procedures.[3]

Principles of the Procedure

Tryptose provides carbon and nitrogen. Dextrose is a carbon source. Sodium Chloride maintains osmotic balance. Buffering capacity is provided by Disodium Phosphate.

The addition of 0.1-0.2% agar to Tryptose Phosphate Broth facilitates anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment.[4] The low agar concentration provides suitable conditions for both aerobic growth in the upper zone and for microaerophilic and anaerobic growth in the lower zone.

Formula

Tryptose Phosphate Broth

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 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

Tryptose Phosphate Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Tubes with closures
Bacto Agar (optional)

Method of Preparation

1. Dissolve 29.5 grams in 1 liter distilled or deionized water.
2. If a medium containing 0.1% agar is desired, add 1 gram of Bacto Agar. Heat to boiling to dissolve completely.
3. Dispense as desired.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

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

Test Procedure

See appropriate references for specific 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.

References

1. **Waisbren, B. A., M. S. Carr, and J. Dunnett.** 1951. The tube dilution method of determining bacterial sensitivity to antibiotics. Am. J. Clin. Pathol. **21**:884.
2. **Ginsberg, Gold, and Jordan.** 1955. Proc. Soc. Exp. Biol. Med. **89**:66.
3. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 802-804. Williams & Wilkins, Baltimore, MD.

Packaging

Tryptose Phosphate Broth	500 g	0060-17
	2 kg	0060-07
	10 kg	0060-08

Bacto® UBA Medium

Intended Use

Bacto UBA Medium is used for cultivating microorganisms of significance in the brewing industry.

Also Known As

UBA is also referred to as Universal Beer Medium or Universal Beer Agar.

Summary and Explanation

UBA Medium is a basal medium to which beer is added. It is based on the formula developed by Kozulis and Page[1] who compared it with other media commonly used in breweries for detecting microbial contamination.[2] The characteristics of UBA Medium are closer to the natural environmental conditions found in the typical brewery than other media studied. UBA Medium supports growth of more varieties of lactic acid bacteria and yields larger colonies in a shorter time than traditional brewer's media. Due to the presence of beer

in the medium, it is selective for growth of microorganisms that have adapted themselves to existent conditions in the brewery. The presence of hop constituents and alcohol inhibits growth of many airborne microorganisms not adapted to this environment.[3]

UBA Medium supports growth of *Lactobacillus*, *Pediococcus*, *Acetobacter* and yeast strains which may be found contaminating the wort and beer.

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins and amino acids. Peptonized Milk contains lactose as an energy source. Tomato Juice is a source of carbon, protein and nutrients. Dextrose provides additional carbon. Dipotassium and Monopotassium Phosphate provide buffering capability. Magnesium Sulfate, Ferrous Sulfate and Manganese Sulfate are sources of ions that stimulate metabolism. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Formula

UBA Medium

Formula Per Liter

Bacto Yeast Extract	6.1 g
Bacto Peptonized Milk	15 g
Tomato Juice (244 ml)	12.2 g
Bacto Dextrose	16.1 g
Dipotassium Phosphate	0.31 g
Monopotassium Phosphate	0.31 g
Magnesium Sulfate	0.12 g
Sodium Chloride	0.006 g
Ferrous Sulfate	0.006 g
Manganese Sulfate	0.006 g
Bacto Agar	12 g

Final pH 6.3 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium beige, homogeneous, free-flowing.
Solution:	6.2% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, very slightly opalescent.
Reaction of 6.2% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Prepare UBA Medium per label directions. Inoculate the medium and incubate at 30 ± 2°C for up to 3 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Acetobacter pasteurianus	12879	100-1,000	good
Lactobacillus fermentum	9338	100-1,000	good
Pediococcus acidilactici	8081	100-1,000	good

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

Precautions

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

Storage

Store 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

UBA Medium

Materials Required but not Provided

Glassware

Autoclave

Method of Preparation

1. Suspend 62 grams in 750 ml distilled or deionized water or halogen-free tap water.
2. Heat to boiling to dissolve completely.
3. Add 250 ml of commercial beer (not degassed) and mix well.
4. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Kozulis, J. A., and H. E. Page.** 1968. A new universal beer agar medium for the enumeration of wort and beer microorganisms. Proc. Am. Soc. Brew. Chem. 1968:52-58.
2. **Murphy, D. T., and L. T. Saletan.** 1970. Use of microbiological media in the brewery. Tech. Q. Master Brew. Assoc. Am. **7**:182-187.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 819-820. Williams & Wilkins, Baltimore, MD.

Packaging

UBA Medium	500 g	0856-17

Bacto® UVM Modified Listeria Enrichment Broth

Intended Use

Bacto UVM Modified Listeria Enrichment Broth is used for rapidly isolating *Listeria monocytogenes*.

Summary and Explanation

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

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

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber with a faint bluish-green ring at the surface, very slightly opalescent with a fine precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare UVM Modified Listeria Enrichment Broth per label directions. Inoculate tubes and incubate at 35 ± 2°C for 18-48 hours.

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

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

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

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

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

UVM Modified Listeria Enrichment Broth is a modification of the formula described by Donnelly and Baigent.[9] It is used for selective enrichment of *Listeria* spp. from food[7,11] and clinical specimens.[10]

Principles of the Procedure

Tryptose, Beef Extract and Yeast Extract in UVM Modified Listeria Enrichment Broth provide nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. Phosphate acts as a buffering agent. Nalidixic acid inhibits growth of gram-negative organisms. Acriflavine hydrochloride inhibits many gram-positive bacteria. Esculin is hydrolyzed by *Listeria* species.

Formula

UVM Modified Listeria Enrichment Broth

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Sodium Chloride	20 g
Sodium Phosphate, Dibasic	9.6 g
Potassium Phosphate, Monobasic	1.35 g
Esculin	1 g
Nalidixic Acid	0.02 g
Acriflavine HCl	0.012 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

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

   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 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

UVM Modified Listeria Enrichment Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Test tubes with closures
Autoclave
Incubator (30°C)
Incubator (35°C)

Method of Preparation

1. Suspend 52 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7,10,11,12]
2. Clinical specimens obtained from nonsterile sites, foods and specimens obtained from the environment should be selectively enriched for *Listeria* species before being plated.[10]
3. Process each specimen using procedures appropriate for that specimen or sample.[7,10,11,12]

Test Procedure

The USDA method[11] involves enrichment of the specimen in UVM Modified Listeria Enrichment Broth (one part sample in nine parts broth) at 30°C. After incubation, a portion of the enrichment mixture is added to an enrichment broth or plated onto the final isolation agar.[7] For further information when testing food samples or clinical specimens, refer to appropriate references.[7,10,11,12]

Results

Refer to appropriate references and procedures for results.

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). J. Path. Bact. **29:**407-439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low-and high-fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma special report. J. Assoc. Off. Anal. Chem. **70**:769-772.
4. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Prot. **58**:604-608.
5. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
6. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
7. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*, p. 637-663. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
9. **Donnelly, C. W., and G. J. Baigent.** 1986. Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl. Environ. Microbiol. **52:**689-695.
10. **Swaminathan, B., J. Rocourt, and J. Bille.** 1995. *Listeria*. *In* P. R. Murray, *et al*. (ed), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
11. **Lee, W. H., and D. McClain.** 1989. *Laboratory Communication No. 57* (revised May 24, 1989). U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
12. **Hayes, P. S., L. M. Graves, B. Swaminathan, G. W. Ajello, G. B. Marcolm, R. E. Weaver, R. Ransom, K. Deaver, B. D. Plikaytis, A. Schuchat, J. D. Wenger, R. W. Pinner, C. V. Broome, and The *Listeria* Study Group.** 1992. Comparison of three selective enrichment methods for the isolation of *Listeria monocytogenes* from naturally contaminated foods. J. Food. Prot. **55:**952-959.

Packaging

UVM Modified Listeria Enrichment Broth	500 g	0223-17
	2 kg	0223-07
	10 kg	0223-08

Bacto® Universal Preenrichment Broth

Intended Use

Bacto Universal Preenrichment Broth is used for recovering sub-lethally injured *Salmonella* and *Listeria* from food products.

Summary and Explanation

Traditional methods for recovering *Salmonella* and *Listeria* from food products require separate preenrichment media for each microorganism.[1, 2]

Some broth media recommended for preenrichment contain antibiotic inhibitors[3] or have insufficient buffering capacity which hinder recovery of sublethally injured cells.[3, 4, 5]

Bailey and Cox[3] formulated Universal Preenrichment Broth to permit simultaneous resuscitation of sublethally injured *Salmonella* and *Listeria*. The broth medium provides sufficient buffering capacity to prevent rapid decreases in pH and allows for repair of injured cells that might be sensitive to low pH values or inhibitory substances.

Principles of the Procedure

Universal Preenrichment Broth contains Tryptone and Proteose Peptone as sources of carbon, nitrogen, vitamins and minerals. Sodium and Potassium Phosphates buffer the medium. Sodium Chloride, Magnesium Sulfate and Ferric Ammonium Citrate provide essential ions. Dextrose is an energy source. Sodium Pyruvate helps stimulate the metabolism of stressed organisms.

Formula

Universal Preenrichment Broth

Formula Per Liter	
Bacto Tryptone	5 g
Bacto Proteose Peptone	5 g
Potassium Phosphate Monobasic	15 g
Sodium Phosphate Dibasic	7 g
Sodium Chloride	5 g
Bacto Dextrose	0.5 g
Magnesium Sulfate	0.25 g
Ferric Ammonium Citrate	0.1 g
Sodium Pyruvate	0.2 g

Final pH 6.3 ± 0.2 at 25°C

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.

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

Universal Preenrichment Broth

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in distilled or deionized water on warming. Solution is light to medium amber, slightly opalescent to opalescent, may have precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent to opalescent, may have precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Listeria monocytogenes	19115	10-100	good to excellent
Salmonella enteritidis	13076	10-100	good to excellent
Salmonella typhimurium	14028*	10-100	good to excellent

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

Salmonella typhimurium ATCC® 14028

Materials Required but not Provided

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

Method of Preparation

1. Suspend 38 grams in 1 liter distilled or deionized water.
2. Heat gently to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Store prepared medium at 2-8°C.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Substitute Universal Preenrichment Broth for preenrichment media as specified for *Salmonella* and *Listeria* [1,2] and follow recommended procedures.

Results

Salmonella and *Listeria* demonstrate good growth and recovery following preenrichment in this broth.

References

1. **Vanderzant, C., and D.F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
3. **Bailey, J. S., and N. A. Cox.** 1992. Universal preenrichment broth for the simultaneous detection of *Salmonella* and *Listeria* in foods. J. Food Protect. **55**:256-259.
4. **Bailey, J. S., D. L. Fletcher, and N. A. Cox.** 1990. Efficacy of enrichment media for recovery of heat-injured *Listeria monocytogenes*. J. Food Prot. **53**:473-477.
5. **Juven, B. J., N. A. Cox, J. S. Bailey, J. E. Thomson, O. W. Charles, and J. V. Shutze.** 1984. Recovery of *Salmonella* from artificially contaminated poultry feed in non-selective and selective broth media. J. Food Prot. **47**:299-302.

Packaging

Universal Preenrichment Broth	500 g	0235-17

Bacto® Urea Agar Base · Bacto Urea Agar Base Concentrate Bacto Urea Broth · Bacto Urea Broth Concentrate

User Quality Control

Identity Specifications

Urea Agar Base

Dehydrated Appearance:	Light orange-red to orange-red, homogeneous, inherently lumpy.
Solution:	2.9% solution, soluble in distilled or deionized water. Solution is orange, clear.
Prepared Agar Medium:	Reddish orange, very slightly opalescent.
Reaction of 2.9% Solution at 25°C:	pH 6.8 ± 0.1

Urea Agar Base Concentrate

Solution:	29% solution is reddish orange, clear liquid.
Reaction of 29% Solution at 25°C:	pH 6.75 ± 0.15

Urea Broth

Dehydrated Appearance:	Light orange to light pink, homogeneous, inherently lumpy.
Solution:	3.87% solution, soluble in distilled or deionized water. Solution is orange-yellow, clear.
Reaction of 3.87% Solution at 25°C:	pH 6.8 ± 0.1

continued on following page

Uninoculated tube | *Proteus vulgaris* ATCC® 13315 | *Escherichia coli* ATCC® 25922

Intended Use

Bacto Urea Agar Base, when combined with Bacto Agar, is used for differentiating microorganisms based on urease activity.

Bacto Urea Agar Base Concentrate is a sterile 10X solution of Urea Agar Base which, when combined with Bacto Agar, is used for preparing Urea Agar.

Bacto Urea Broth is used for differentiating microorganisms, particularly *Proteus* species, based on urease production.

Bacto Urea Broth Concentrate is a sterile 10X solution of Urea Broth ready to use as recommended. It is suggested for laboratories that require only small amounts of medium.

Also Known As

Urea Agar Base is also known as Urea Agar Base, Christensen or Christensen's Urea Agar.

Urea Broth is also referred to as Stuart's Urea Broth.

Summary and Explanation

Christensen[1] devised a urea agar medium containing peptone and dextrose that had a reduced buffer content. The medium supported a more vigorous growth of many of the gram-negative enteric bacilli and readily permitted observation of urease production.

Ewing[2] used Urea Agar as a differential medium in the examination of many cultures from stool specimens. Urea Agar may be used as a screening medium (along with Triple Sugar Iron Agar) for the selection of *Salmonella* and *Shigella* cultures for serologic classification.[3] Qadri et al.[4] developed a spot test for the rapid detection of urease activity by applying diluted Urea Agar Base Concentrate to filter paper and inoculating the paper with a loopful of 24-48 hour culture. Urease-positive results were obtained within 2 minutes. When combined with results of other rapid screening methods, Urea Agar is the most common way to detect the production of urease by yeasts.[5] Urea Agar Base Concentrate has also been used in differentiating mycobacteria species.[6]

Urea Broth, prepared according to the formula of Stuart, Van Stratum and Rustigian[7] is a highly buffered urea medium that provides all the essential growth requirements for *Proteus*. Stuart et al.[7] noted that by decreasing the amount of buffer in their standard medium to one-tenth or one-hundredth of the original concentration, the incubation time for *Proteus* could be decreased from 12-48 hours to 2-4 hours. When the amount of buffer is decreased, however, other organisms capable of urease production give a positive test. Rustigian and Stuart[8] used urea decomposition as a limiting characteristic for the identification of *Proteus* strains from other members of the family *Enterobacteriaceae*. Ferguson and Hook[9] reported that urease production could be used to differentiate between members of the *Proteus* and *Salmonella* groups. The medium is positive for *Proteus*, *Morganella morganii*, *Providencia rettgeri* and a few *Providencia stuartii* strains.

The detection of urease production is an important differential test in microbiology and is outlined in standard references.[10-16]

Principles of the Procedure

Bacto Peptone provides carbon and nitrogen required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth and as an additional source of nitrogen and carbon. Dextrose is included as an energy source. Sodium Chloride maintains the osmotic balance of the medium. Potassium Phosphate, Monobasic and Potassium Phosphate, Dibasic provide buffering capability. Urea provides a source of nitrogen for those organisms producing urease. This is indicated by a color change of the pH indicator, Phenol Red, from yellow (pH 6.8) to red to pink-red (pH 8.1).

User Quality Control cont.

Urea Broth Concentrate

Appearance:	38.7% solution is reddish-orange, clear liquid.
Reaction of 38.7% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Urea Agar Base and Urea Agar Base Concentrate

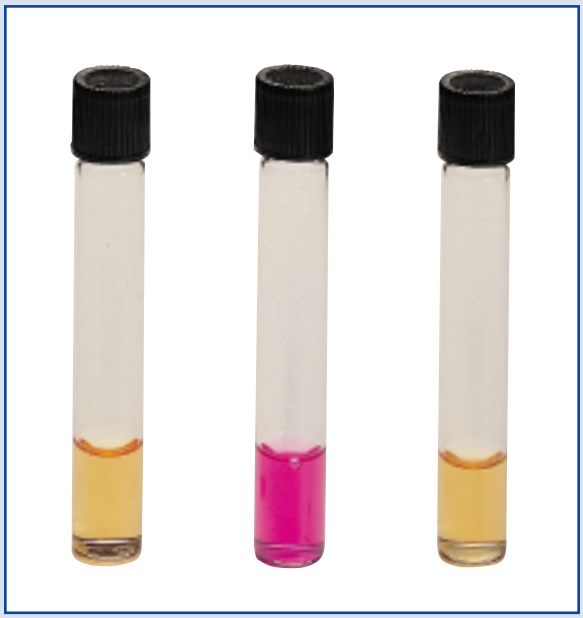
Prepare Urea Agar per label directions. Inoculate and incubate at 35 ± 2°C for 6-48 hours.

Urea Broth and Urea Broth Concentrate

Prepare Urea Broth per label directions. Inoculate and incubate at 35 ± 2°C for 8-48 hours.

ORGANISM	ATCC®	UREASE PRODUCTION
Escherichia coli	25922*	negative, no color change in the medium
Proteus vulgaris	13315*	positive, red or cerise medium

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



Uninoculated tube | *Proteus vulgaris* ATCC® 13315 | *Escherichia coli* ATCC® 25922

Urea Broth

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

Formula

Urea Agar Base

Formula Per Liter

Bacto Peptone	1 g
Bacto Dextrose	1 g
Sodium Chloride	5 g
Potassium Phosphate, Monobasic	2 g
Urea	20 g
Bacto Phenol Red	0.012 g

Final pH 6.8 ± 0.1 at 25°C

Urea Agar Base Concentrate

A liquid, 10X concentrate of Urea Agar Base.

Urea Broth

Formula Per Liter

Bacto Yeast Extract	0.1 g
Potassium Phosphate, Monobasic	9.1 g
Potassium Phosphate, Dibasic	9.5 g
Bacto Urea	20 g
Bacto Phenol Red	0.01 g

Final pH 6.8 ± 0.1 at 25°C

Urea Broth Concentrate

A liquid, 10X concentrate of Urea Broth.

Precautions

1. For Laboratory Use.
2. **Urea Broth**: **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 media at 2-8°C. The dehydrated media are very hygroscopic. Keep containers tightly closed. Store the prepared media also at 2-8°C.

Store Urea Agar Base Concentrate and Urea Broth Concentrate at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Urea Agar Base
Urea Agar Base Concentrate
Urea Broth
Urea Broth Concentrate

Materials Required But Not Provided

Glassware
Autoclave
Refrigerator (2-8°C)
Waterbath (50-55°C) (optional)
Incubator (35°C)
Bacto Agar
Filter sterilization apparatus

Method of Preparation

Urea Agar Base

Equilibrate this medium to room temperature before opening.

The presence of urea in this medium renders it inherently lumpy. This condition will not adversely affect a properly stored medium.

1. Dissolve 29 grams in 100 ml distilled or deionized water.
2. Filter sterilize. DO NOT BOIL OR AUTOCLAVE.
3. Suspend 15 grams of Bacto Agar in 900 ml distilled or deionized water.
4. Boil to dissolve completely.
5. Autoclave at 121°C for 15 minutes.
6. Cool to 50-55°C.
7. Aseptically add 100 ml of the filter sterilized Urea Agar Base to the cooled Bacto Agar. Mix thoroughly. DO NOT HEAT THE COMPLETE MEDIUM.
8. Distribute in sterile test tubes. Slant the tubes to have a butt about 2 cm in depth and a slant about 3 cm in length.

Urea Agar Base Concentrate

If crystals have formed in the concentrate prior to preparing the final medium, place the tube(s) in a water bath at 40-50°C for a few moments. Agitate to dissolve the crystals.

1. Suspend 1.5 grams of Bacto Agar in 90 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50-55°C. Aseptically add 10 ml of Urea Agar Base Concentrate.
5. Mix thoroughly; dispense into tubes and slant.

Urea Broth

Equilibrate this medium to room temperature before opening.

The presence of urea in this medium renders it inherently lumpy. This condition will not adversely affect a properly stored medium.

1. Dissolve 38.7 grams in 1 liter distilled or deionized water. Mix thoroughly to dissolve completely.
2. Filter sterilize. DO NOT BOIL OR AUTOCLAVE THE MEDIUM.
3. Aseptically distribute 3 ml amounts into sterile test tubes (14 x 125 mm or equivalent).

Urea Broth Concentrate

If crystals have formed in the concentrate prior to preparing the final medium, place the tube(s) in a water bath at 40-50°C for a few moments. Agitate to dissolve the crystals.

Do no heat Urea Broth above 50°C during preparation or sterilization.

1. To prepare 100 ml of final medium, sterilize 90 ml of distilled or deionized water at 121-124°C for 15 minutes.
2. Cool to 50-55°C. Aseptically add 10 ml of Urea Broth Concentrate. Mix thoroughly.
3. Distribute 3 ml amounts into sterile test tubes (14 x 125 mm).

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Urea Agar

1. Use a heavy inoculum of growth from a pure 18-24 hour culture. Inoculate by streaking back and forth over the entire slant surface. Do not stab the butt because it serves as a color control.
2. Incubate tubes with loosened caps at 35 ± 2°C.
3. Observe reactions after 6 and 24 hours and every day thereafter for a total of 6 days.[1] Longer periods of incubation may be necessary.

Urea Broth

1. Inoculate with a heavy inoculum, using a straight needle or a drop from an 18-24 hour culture. Shake tube gently to resuspend the bacteria.
2. Incubate aerobically at 35 ± 2°C.
3. Record reactions after 8, 12, 24 and 48 hours of incubation.

Results

Urea Agar

Positive: The production of urease is indicated by an intense red or cerise color on the slant which may penetrate into the butt.

Negative: No color change of the medium.

Urea Broth

Positive: The production of urease is indicated by an intense red or cerise color throughout the broth.

Negative: No color change of the broth.

Limitations of the Procedure

Urea Agar Base

1. The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. False positive reactions may occur due to the utilization of peptones (especially in slant agar by *Pseudomonas aeruginosa*, for example) or other proteins which raise the pH due to protein hydrolysis and the release of excessive amino acid residues. To eliminate possible protein hydrolysis, perform a control test with the same test medium without urea.[17]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. Urea Agar detects rapid urease activity of only the urease-positive *Proteus* species. For results to be valid for the detection of *Proteus*, the results must be read within the first 2 to 6 hours after incubation. Urease-positive *Enterobacter, Citrobacter* or *Klebsiella*, in contrast, hydrolyze urea much more slowly, showing only slight penetration of the alkaline reaction into the butt of the medium in 6 hours and requiring 3 to 5 days to change the reaction of the entire butt.

Urea Broth

1. To rule out false positives due to protein hydrolysis (as opposed to urea hydrolysis) that may occur in the medium after prolonged incubation, perform a control test with the same test medium without urea.[17]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. The high buffering system in this medium masks urease activity in organisms that are delayed positive. This medium is therefore recommended for the detection of urease activity in all *Proteus* spp., *Providencia rettgeri* and urease- positive *Providencia stuartii*.[1] *M. morganii* slowly hydrolyzes urea and may require approximately a 36 hour incubation for a strong urease-positive reaction to occur.[1] If in doubt as to a result, compare with an uninoculated tube or incubate for an additional 24 hours.
4. Variations in the size of the inoculum can affect the time required to reach positive (alkaline, pH 8.1) results. The accepted standard inoculum is 0.1 ml.[1]

References

1. **Christensen, W. B.** 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. J. Bacteriol. **52**:461.
2. **Ewing, W. H.** 1946. An additional *Shigella paradysenteriae* serotype. J. Bacteriol. **51**:433-445.
3. **Ewing, W. H., and D. W. Bruner.** 1947. Selection of *Salmonella* and *Shigella* cultures for serologic classification. Am. J. Clin. Path. **17**:1-12.
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5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th edition. Mosby-Year Book, Inc., St. Louis, MO.
6. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology - A guide for the level III laboratory. U.S. Public Health Service, Atlanta, GA.
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9. **Ferguson, W. W., and A. E. Hook.** 1943. Urease activity of *Proteus* and *Salmonella* organisms. J. Lab. Clin. Med. **28**:1715-1719.
10. **Vanderzant, C., and D. F. Splittstoesser.** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Assoc., Washington, D.C.
11. **Marshall, R. T. (ed.)** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Assoc., Washington, D.C.
12. **Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams.** 1994. Bergey's manual of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, MD.

13. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken.** 1995. Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
14. **Bacteriological Analytical Manual,** 8th ed. 1995. AOAC International, Gaithersburg, MD.
15. **Oberhofer, T. R.** 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, NY.
16. **Ewing, W. H.** 1986. Edwards and Ewing's Identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, NY.
17. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Urea Agar Base	100 g	0283-15
	500 g	0283-17
Urea Agar Base Concentrate 10X	12 x 10 ml	0284-61
Urea Broth	500 g	0272-17
Urea Broth Concentrate	12 x 10 ml	0280-61

Bacto® VJ Agar

Intended Use

Bacto VJ Agar is used with Bacto Chapman Tellurite Solution 1% for isolating coagulase-positive, mannitol-fermenting staphylococci.

Also Known As

VJ Agar is also known as Vogel and Johnson Agar, Modification of Tellurite-Glycine Agar[1], and Tellurite-Glycine-Phenol Red Agar Base[2]

Summary and Explanation

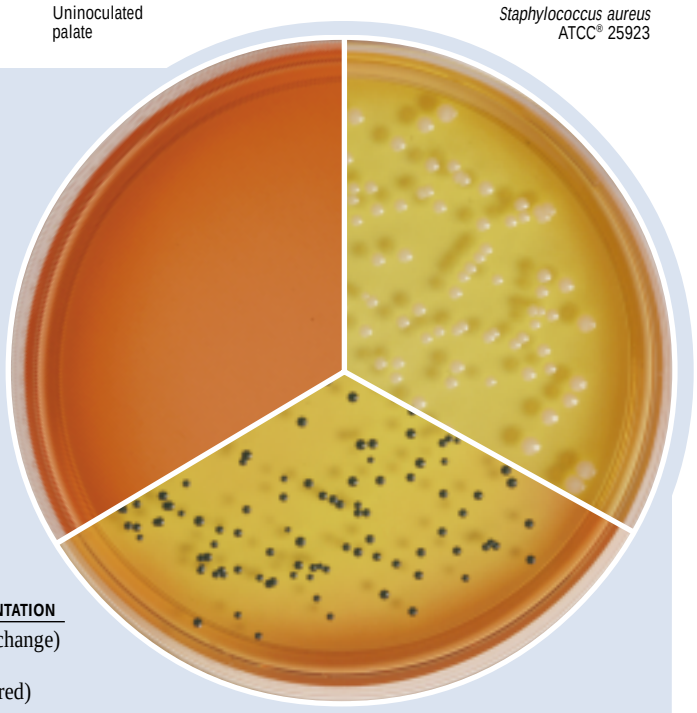
Coagulase-positive staphylococci, primarily *Staphylococcus aureus*, are among the microorganisms that can cause spoilage or chemical changes in cosmetic products.[4]

To isolate coagulase-positive, mannitol fermenting staphylococci, Vogel and Johnson[3] modified Tellurite-Glycine Agar by Zebovitz et al.[1] by increasing the mannitol content and adding a pH indicator. Vogel-Johnson (VJ) Agar selects and differentiates the coagulase-positive staphylococci which ferment mannitol and reduce tellurite.[2]

VJ Agar is specified as a standard methods medium for cosmetics,[4,5] pharmaceutical articles[6] and nutritional supplements.[6]

Principles of the Procedure

VJ Agar contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Mannitol is the carbohydrate. Chapman



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, homogenous, free-flowing.
Solution:	6.0% solution, soluble in distilled or deionized water on boiling. Solution is red, slightly opalescent with a white precipitate.
Prepared Medium:	Red, slightly opalescent, may have slight white precipitate.
Reaction of 6.0% Solution at 25°C:	pH 7.2 ± 0.1

Cultural Response

Prepare VJ Agar per label directions with the addition of Chapman Tellurite Solution, 1%. Inoculate and incubate the plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	TELLURITE GROWTH	MANNITOL REDUCTION	MANNITOL FERMENTATION
Escherichia coli	25922*	marked to complete inhibition	– (no change)	– (no change)
Proteus mirabilis	25933	partial to complete inhibition	+ (black)	– (red)
Staphylococcus aureus	25923*	good	+ (black)	+ (yellow)
Staphylococcus epidermidis	12228*	none to fair	± (translucent to black)	– (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.

Tellurite Solution 1% contains Potassium Tellurite which, along with Lithium Chloride and Glycine, inhibits most microorganisms except the staphylococci. Phenol Red is the pH indicator. Bacto Agar is the solidifying agent.

Formula

Bacto VJ Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Mannitol	10 g
Dipotassium Phosphate	5 g
Lithium Chloride	5 g
Glycine	10 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.2 ± 0.1 at 25°C

Precautions

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

VJ Agar

Materials Required but not Provided

Chapman Tellurite Solution 1%
Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

VJ Agar

1. Suspend 60 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. Add 20 ml Chapman Tellurite Solution 1%. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Coagulase-positive strains of *S. aureus* reduce tellurite and form black colonies on the medium. These strains typically ferment mannitol and exhibit yellow halos around the black colonies.

References

1. **Zebovitz, E., J. B. Evans, and C. F. Niven, Jr**. 1955. Tellurite-Glycine Agar: a selective plating medium for the quantitative detection of coagulase-positive staphylococci. J. Bacteriol. **70**:686.
2. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 846-849. Williams & Wilkins, Baltimore, MD.
3. **Vogel, R. A., and M. Johnson**. 1960. A modification of the Tellurite-Glycine medium for use in the identification of *Staphylococcus aureus*. Public Health Lab. **18**:131.
4. **Hitchins, A. D., T. T. Tran, and J. E. McCarron**. 1995. Microbiological methods for cosmetics, p. 23.01-23.11. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (ed.)**. 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
6. **United States Pharmacopeial Convention**. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.

Packaging

VJ Agar	100 g	0562-15
	500 g	0562-17

Bacto® Veal Infusion Agar
Bacto Veal Infusion Broth

Intended Use

Bacto Veal Infusion Agar is used for cultivating fastidious microorganisms with or without added enrichment.

Bacto Veal Infusion Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

The nutritive factors of Veal Infusion media permit luxuriant growth of fastidious microorganisms. Veal Infusion Agar may be used as a base with blood, ascitic fluid, serum or other enrichments. Veal Infusion media are specified for use in the examination of food.[1,2] Veal Infusion Agar is specified in AOAC Official Methods of Analysis for culturing eggs and egg products, and as a maintenance medium for *E. coli*.[3] Veal Infusion Broth is recommended for culturing *E. coli* in the AOAC procedure for invasiveness of mammalian cells.[3]

User Quality Control

Identity Specifications

Veal Infusion Agar

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in distilled or deionized water on boiling. Light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent without precipitate.
Reaction of 4.0% Solution at 25°C:	pH 7.4 ± 0.2

Veal Infusion Broth

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	2.5% solution, soluble in distilled or deionized water, very light amber, clear to very slightly opalescent.
Prepared Medium:	Very light amber, clear to very slightly opalescent with no more than very slight precipitation.
Reaction of 2.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Veal Infusion Agar per label directions with and without 5% sterile defibrinated sheep blood. Inoculate medium with the test organisms. Incubate inoculated plates at 35 ± 2°C for 18-48 hours under approximately 10% CO_2.

Prepare Veal Infusion Broth per label directions. Inoculate tubes with the test organisms. Incubate inoculated tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	good
Staphylococcus epidermidis	12228*	100-1,000	good
Streptococcus mitis	9895	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good

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

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

Principles of the Procedure

Infusion from Lean Veal and Proteose Peptone No. 3 provides the nitrogen, vitamins, carbon and amino acids in Veal Infusion media. Sodium Chloride maintains the osmotic balance of the formulations. Bacto Agar is the solidifying agent in Veal Infusion Agar.

Formula

Veal Infusion Agar

Formula Per Liter

Lean Veal, Infusion from	500 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Veal Infusion Broth

Formula Per Liter

Lean Veal, Infusion from	500 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	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

Veal Infusion Agar
Veal Infusion Broth

Materials Required But Not Provided

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

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:
   Veal Infusion Agar 40 g/l
   Veal Infusion Broth 25 g/l
2. Heat to boiling to dissolve completely (Veal Infusion Agar).
3. Autoclave at 121°C for 15 minutes.

4. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense as desired.

Specimen Collection and Preparation

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

Test Procedure

For a complete discussion on the examination of fastidious microorganisms in food refer to the procedures outlined in the references.[1,2,3]

Limitations of the Procedure

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

References

1. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, 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. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Veal Infusion Agar	500 g	0343-17
Veal Infusion Broth	500 g	0344-17
	10 kg	0344-08

Bacto® Veillonella Agar

Intended Use

Bacto Veillonella Agar is used with added vancomycin in isolating *Veillonella*.

Summary and Explanation

Veillonella Agar is prepared according to the formula described by Rogosa[1,2] as modified by Rogosa, Fitzgerald, MacKintosh and Beaman.[3] Rogosa's[2] experiments with oral specimens from humans and rats demonstrated the medium to be highly selective for *Veillonella* species. Streptomycin was originally employed as the selective agent. Later, Rogosa et al.[3] demonstrated vancomycin to be superior to streptomycin in reducing growth of extraneous organisms without restricting growth of *Veillonella*.

Veillonella parvula is part of the normal human fecal flora.[4] *V. parvula*, *V. atypica* and *V. dispar* are flora colonizing the oral cavity.[4] *Veillonella* species have been encountered in patients with bite wound, head, neck, oral and miscellaneous soft tissue infections.[5] *Veillonella* species are anaerobic gram negative diplococci and appear as clumps of diplococci when stained.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous with small dark particles.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling.
Prepared Medium:	Pink, slightly opalescent without precipitate.
Reaction of 3.6% Solution at 25°C	pH 7.5 ± 0.2

Cultural Response

Prepare Veillonella Agar per label directions. Using the pour plate technique, inoculate plates with 1 ml of the diluted test organisms and 1 ml of the specimen. Pour 20 ml medium per plate, mix well. Incubate plates at 35 ± 2°C anaerobically for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Veillonella criceti	17747	100-1,000	good
Veillonella dispar	17748	100-1,000	good
Veillonella ratti	17746	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	inhibited

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

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

Principles of the Procedure

Tryptone and Yeast Extract provide the nitrogen, vitamins, amino acids and carbon in Veillonella Agar. Sodium Thioglycollate is a reducing agent, and lowers the oxidation-reduction potential of the medium by removing oxygen to maintain a low pH. Basic Fuchsin and Vancomycin are the selective agents. Sodium Lactate, 60% provides nutrients and selective properties. Bacto Agar is the solidifying agent.

Formula

Veillonella Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	3 g
Sodium Thioglycollate	0.75 g
Bacto Basic Fuchsin	0.002 g
Sodium Lactate, 60%	21 ml
Bacto Agar	15 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 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

Veillonella Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes
Tween® 80 (optional)
Vancomycin

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. OPTIONAL: Add 1 gram Tween® 80, if desired.
4. Autoclave at 121°C for 15 minutes.
5. Add 7.5 mcg vancomycin per ml of sterile medium at 50-55°C. Mix thoroughly.

Specimen Collection and Preparation

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[6] Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

1. Rogosa[1,2,3] recommends that one ml of the diluted specimen be added to a sterile Petri dish.
2. Pour approximately 20 ml of medium to the Petri dish, and rotate to mix well with the inoculum.
3. Incubate plates anaerobically at 35 ± 2°C for 40-48 hours; 72 hours if necessary.

For a complete discussion on *Veillonella* species from clinical specimens, refer to the appropriate procedures outlined in the references.[4,6,7] For the examination of anaerobic bacteria in food refer to standard methods.[8, 9, 10]

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.[6]
3. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[6]
4. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure the organism is an anaerobe.[6]

References

1. **Rogosa, M.** 1955. Nutrition of the *Vellonella*. J. Dent. Res. **34**:721-722.
2. **Rogosa, M.** 1956. A selective medium for the isolation and enumeration of the *Veillonella* from the oral cavity. J. Bacteriol. **72**:533-536.
3. **Rogosa, M., R. J. Fitzgerald, M. E. MacKintosh, and A. J. Beaman.** 1958. Improved medium for selective isolation of *Veillonella*. J. Bacteriol. **76**:455-456.
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. **Summanen, P., E. J. Baron, D. M. Citron, C. Strong, H. M. Wexler, and S. M. Finegold.** 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing Co., Belmont, CA.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **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.
8. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
9. **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.
10. **Marshall, R. T. (ed.).** 1992. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington. D.C.

Packaging

Veillonella Agar	500 g	0917-17

Bacto® Violet Red Bile Agar

Intended Use

Bacto Violet Red Bile Agar is used for enumerating coliform organisms in dairy products.

Also Known As

Violet Red Bile Lactose Agar

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 the group but other lactose fermenting organisms may also be included.

Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products.[1,2,3] One method for performing the presumptive test for coliforms uses Violet Red Bile Agar (VRBA). If typical coliform colonies appear, they are tested further to confirm their identification as coliforms.

Principles of the Procedure

Violet Red Bile Agar (VRBA) contains Bacto Peptone to provide carbon and nitrogen sources for general growth requirements. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Bile Salts No. 3 and Crystal Violet inhibit most gram-positive microorganisms. Lactose is the carbohydrate source and Neutral Red is the pH indicator. Bacto Agar is the solidifying agent.

Formula

Violet Red Bile Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Peptone	7 g
Bacto Bile Salts No. 3	1.5 g
Bacto Lactose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g
Neutral Red	0.03 g
Bacto Crystal Violet	0.002 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Violet Red Bile Agar

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Incubator (35°C or 32°C for dairy products)

Method of Preparation

1. Suspend 41.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Do not boil for more than 2 minutes. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Presumptive test for coliforms using solid medium:

1. Transfer a 1 ml aliquot of test sample to a Petri dish.
2. Add 10 ml of Violet Red Bile Agar (at 48°C) and swirl to mix.
3. Allow medium to solidify before incubating at 35°C for 18 to 24 hours; use 32°C for dairy products.
4. Examine for purple-red colonies, 0.5 mm in diameter (or larger), surrounded by a zone of precipitated bile acids.
5. Continue with confirmatory testing of typical coliform colonies.[1,2,3]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Reddish-beige, homogeneous, free-flowing.
Solution:	4.15% solution, reddish-purple, very slightly to slightly opalescent.
Prepared plates:	Reddish-purple, slightly opalescent.
Reaction of 4.15% solution at 25°C:	7.4 ± 0.2

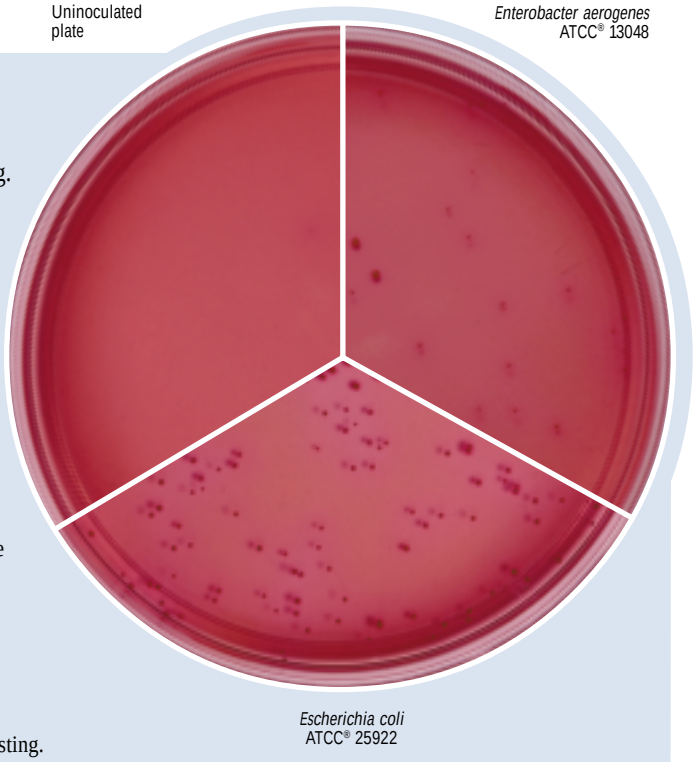
Cultural Response

Prepare Violet Red Bile Agar per label directions. Inoculate and incubate the plates at 32 ± 1°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Enterobacter aerogenes	13048*	30-300	good	red, may have slight red precipitate around colonies
Escherichia coli	25922*	30-300	good	deep red with red precipitate around colonies
Staphylococcus aureus	25923*	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 Disks Technical Information.



Results

Lactose fermenters: Purple-red colonies, with or without a zone of precipitate around the colonies

Lactose non-fermenters: Colorless to transparent colonies

Gram-positive cocci: Colorless, pinpoint colonies

Limitations of the Procedure

1. Violet Red Bile Agar may not be completely inhibitory to gram-positive organisms. Perform Gram stain and biochemical tests as necessary to identify isolates.
2. The medium will grow gram-negative bacilli other than members of the *Enterobacteriaceae*. Perform biochemical tests to identify isolates to genus and species.
3. Boiling the medium for longer than 2 minutes can decrease the ability to support growth.
4. Plates of Violet Red Bile Agar should not be incubated longer than 24 hours because microorganisms that are only partially inhibited may grow after extended incubation.
5. For optimum performance, prepare and use the medium within 24 hours.

References

1. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1993. Coliform and other indicator bacteria, p. 247-252. *In* Marshall, R. T. (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms - *Escherichia coli* and its toxins, p. 325-369. *In* Vanderzant, C., and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **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.

Packaging

Violet Red Bile Agar	100 g	0012-15
	500 g	0012-17
	2 kg	0012-07

Bacto® Violet Red Bile Agar with MUG

Intended Use

Bacto Violet Red Bile Agar with MUG is used for enumerating *Escherichia coli* and total coliform bacteria in food and dairy products.

Also Known As

VRBA with MUG

Summary and Explanation

Violet Red Bile Agar is specified in Standard Methods procedures to enumerate coliforms in food and dairy products.[1,2,3] In 1982, Feng and Hartman developed a rapid fluorogenic assay for *Escherichia coli* by incorporating 4-methylumbelliferyl-ß-D-glucuronide (MUG) into Lauryl Tryptose Broth.[4] Incorporating MUG into Violet Red Bile Agar permits the detection of *E. coli* among the coliform colonies.[2, 3]

Standard Methods procedures specify Violet Red Bile Agar with MUG for detecting *E. coli* in food and dairy products by fluorescence.[1,2,3]

Principles of the Procedure

Violet Red Bile Agar contains Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Bile Salts No. 3 and Crystal Violet inhibit gram-positive bacteria. Lactose is a carbohydrate source. Neutral Red is a pH indicator. MUG (4-methylumbelliferyl-ß-D-glucuronide) is a substrate used for detecting glucuronidase activity. Bacto Agar is a solidifying agent.

E. coli produces the enzyme glucuronidase which hydrolyzes MUG to yield a fluorogenic compound detectable with long-wave UV light (366 nm). Typical strains of *E. coli* (red colonies surrounded by a bile precipitate) exhibit blue fluorescence. Non-*E. coli* coliforms may produce red colonies with zones of precipitated bile but they are MUG negative.

Formula

Violet Red Bile Agar with MUG

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Peptone	7 g
Bacto Bile Salts No. 3	1.5 g
Bacto Lactose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g
Neutral Red	0.03 g
Bacto Crystal Violet	0.002 g
MUG (4-methylumbelliferyl-ß-D-glucuronide)	0.1 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

Violet Red Bile Agar with MUG

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (32°C)
Waterbath (45°C)

Method of Preparation

1. Suspend 41.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling and boil no more than 2 minutes to dissolve completely. DO NOT AUTOCLAVE.
3. Cool to 45°C.
4. 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.[1,2,3]

Test Procedure

1. Process each specimen as appropriate for that specimen.[1,2,3]
2. Incubate plates at 35°C for 22-26 hours.
3. Examine plates for growth and fluorescence.

Results

Coliform organisms form purplish-red colonies that are generally surrounded by a reddish zone of precipitated bile. When examined under long-wave fluorescent light, MUG-positive colonies are surrounded by a bluish fluorescent halo. MUG-negative colonies lack the fluorescent halo.

E. coli colonies are red surrounded by a zone of precipitated bile and fluoresce blue under long-wave UV light.

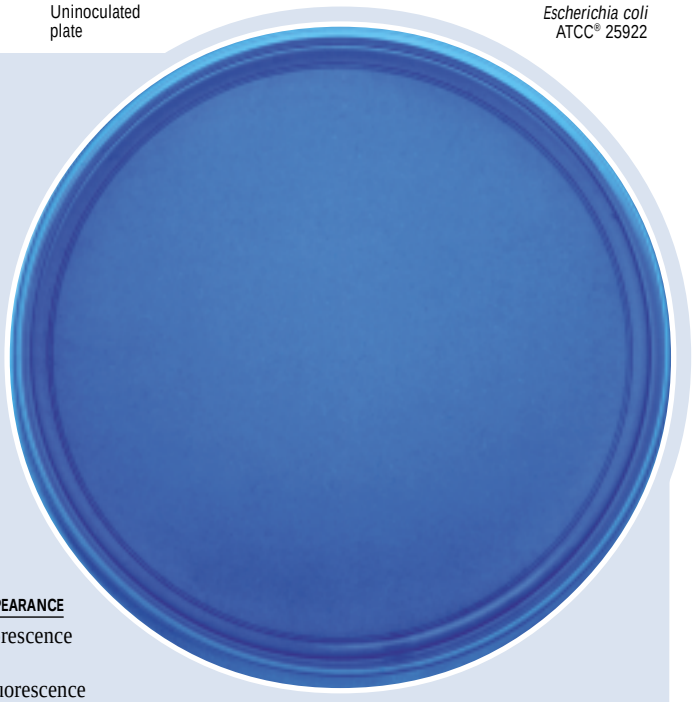
Salmonella and *Shigella* strains that produce glucuronidase may be encountered infrequently but these are generally lactose negative and appear as colorless colonies which may fluoresce.

Limitations of the Procedure

1. Glucuronidase-negative strains of *E. coli* have been encountered.[5,6,7] Similarly, glucuronidase-positive strains of *E. coli* that do not fluoresce have been reported.[8]
2. Strains of *Salmonella* and *Shigella* that produce glucuronidase may infrequently be encountered.[9] These strains must be distinguished from *E. coli* on the basis of other parameters, e. g., gas production, lactose fermentation or growth at 44.5°C.
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. **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.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Reddish beige, free-flowing, homogeneous.
Solution:	4.16% solution, soluble in distilled or deionized water on boiling. Solution is reddish purple, slightly opalescent, without significant precipitate. Very slight surface material may be present.
Prepared Medium:	Reddish purple, very slightly to slightly opalescent, no significant precipitate.
Reaction of 4.16% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Violet Red Bile Agar with MUG per label directions. Inoculate and incubate at 32 ± 2°C for 22-26 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR	APPEARANCE
Escherichia coli	25922*	30-300	good	deep red, with a bile ppt.	fluorescence
Enterobacter aerogenes	13048*	30-300	good	pink, may have a bile ppt.	no fluorescence
Staphylococcus aureus	25923*	1,000	marked to complete inhibition	–	–

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

2. **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.
3. **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.
4 **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43**:1320-1329.
5. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of ß-D-glucuronidase- negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.
6. **Hansen, W., and E. Yourassowsky.** 1984. Detection of ß-glucuronidase in lactose fermenting members of the family Enterobacteriaceae and its presence in bacterial urine cultures. J. Clinical Microbiol. **20**:1177-1179.
7. **Kilian, M., and P. Bulow.** 1976. Rapid diagnosis of Enterobacteriaceae. Acta Pathol. Microbiol. Scand. Sect. B **84**:245-251.
8. **Mates, A., and M. Shaffer.** 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriology **67**:343-346.
9. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. Journal of Food Science **50**:1736-1746.

Packaging

Violet Red Bile Agar with MUG	500 g	0029-17

Bacto® Violet Red Bile Glucose Agar

Intended Use

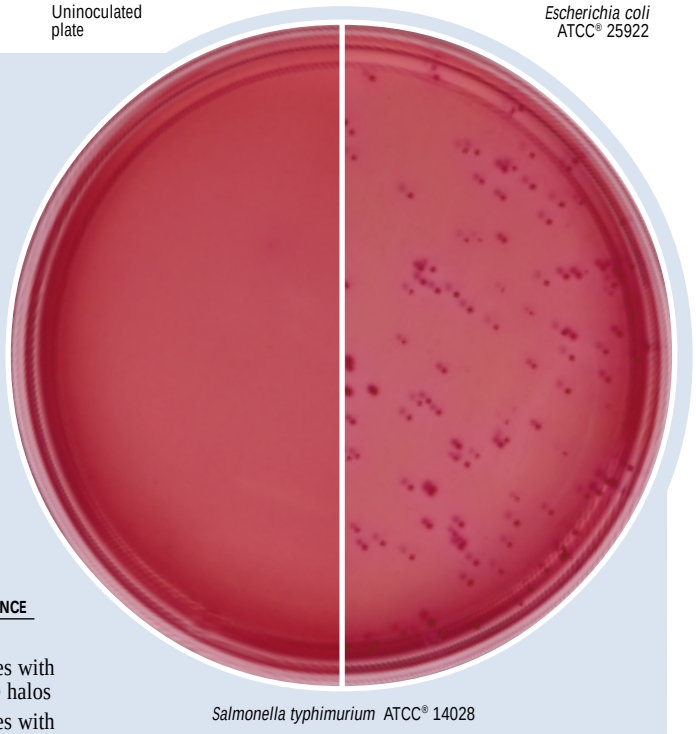
Bacto Violet Red Bile Glucose Agar is used for detecting and enumerating *Enterobacteriaceae* in foods and dairy products.

Also Known As

Violet Red Bile Glucose Agar is also known as VRBGA.

Summary and Explanation

The *Enterobacteriaceae* group includes lactose-fermenting coliform bacteria, nonlactose-fermenting strains of *E. coli*, and nonlactose-fermenting species such as *Salmonella* and *Shigella*. When examining



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink-beige, free-flowing, homogeneous.
Solution:	4.15% solution, soluble in distilled or deionized water on boiling. Solution is reddish-purple, very slightly to slightly opalescent, without significant precipitate.
Prepared Medium:	Reddish-purple, very slightly to slightly opalescent.
Reaction of 4.15% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Violet Red Bile Glucose Agar per label directions. Using the pour plate method, inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Acinetobacter baumanii	19606	1,000-2,000	none to poor	–
Escherichia coli	25922*	30-300	good	red colonies with red-purple halos
Salmonella typhimurium	14028*	30-300	good	red colonies with red-purple halos
Staphylococcus aureus	25923*	1,000-2,000	none to poor	–

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

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

some foods, it is desirable to detect *Enterobacteriaceae* rather than the coliform bacteria.[1,2]

Enterobacteriaceae are glucose-fermenting bacteria. Mossel et al[3] modified Violet Red Bile Agar, suggested by MacConkey[4], that contains lactose by adding glucose to improve the recovery of *Enterobacteriaceae*. Later work by Mossel et al.[5,6] demonstrated that lactose could be omitted, resulting in the formulation known as Violet Red Bile Glucose Agar.

Principles of the Procedure

Violet Red Bile Glucose Agar contains Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Glucose is a carbohydrate. Bile Salts No. 3 and Crystal Violet inhibit gram positive bacteria. Glucose fermenters produce red colonies with red-purple halos in the presence of Neutral Red, a pH indicator. Bacto Agar is a solidifying agent.

Formula

Violet Red Bile Glucose Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Peptone	7 g
Bacto Bile Salts No. 3	1.5 g
Glucose	10 g
Sodium Chloride	5 g
Neutral Red	0.03 g
Bacto Crystal Violet	0.002 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. 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.

   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

Violet Red Bile Glucose Agar

Materials Required but not Provided

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

Method of Preparation

1. Suspend 41.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling and boil for no more than 2 minutes to dissolve completely.
3. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

This medium can be used in spread or pour plate procedures, with or without an overlay. In addition, this medium can be used as an overlayer for spread plates to both prevent swarming colonies and to provide semi-anaerobic conditions that suppress the growth of nonfermentative gram negative organisms. Stab inoculation procedures can also be used with this medium.

Results

Enterobacteriaceae ferment glucose, produce acid products and form red to dark purple colonies surrounded by red-purple halos.

Limitations of the Procedure

1. When used in the pour plate procedure, the medium should be freshly prepared, tempered to 47°C, and used within 3 hours.

References

1. Draft Standard Methods for Microbiological Examination of Meat Products. Part 3: Detection and enumeration of Enterobacteriaceae. BS5393: Part 3 1977 ISO/DIS 5552.
2. **Mossel, D. A. A.** 1985. Media for *Enterobacteriaceae.* Int. J. Food Microbiol. **2**:27.
3. **Mossel, D. A. A., W. H. J. Mengerink, and H. H. Scholts.** 1962. Use of a modified MacConkey agar medium for the selective growth and enumeration of *Enterobacteriaceae.* J. Bacteriol. **84**:381.
4. **MacConkey, A.** 1905. Lactose-fermenting bacteria in faeces. J. Hyg. **5**:333-378.
5. **Mossel, D. A. A., I. Eelderink, M. Koopmans, and F. van Rossem.** 1978. Lab Practice **27**:1049-1050.
6. **Mossel, D. A. A., I. Eelderink, M. Koopmans, and F. van Rossem.** 1979. Influence of carbon source, bile salts and incubation temperature on recovery of *Enterobacteriaceae* from foods using macconkey-type agars. J. Food Protect. **42**:470-475.

Packaging

Violet Red Bile Glucose Agar	500 g	1866-17

Bacto® Vitamin B_{12} Assay Medium

Intended Use

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

Summary and Explanation

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

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.

Vitamin B_{12} Assay Medium is prepared according to the formula described by Capp, Hobbs and Fox.[1] This medium is used in the microbiological assay of vitamin B_{12} using *Lactobacillus delbrueckii* subsp. *lactis* ATCC® 4797 or 7830 (*Lactobacillus leichmannii*).

Principles of the Procedure

Vitamin B_{12} Assay Medium is a vitamin B_{12}-free medium containing all other nutrients and vitamins essential for the cultivation of *L. delbrueckii* subsp. *lactis* ATCC® 4797 or 7830. To obtain a standard curve, USP Cyanocobalamin Reference is added in specified increasing concentrations providing a growth response that can be measured titrimetrically or turbidimetrically.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light to light beige, homogeneous, with a tendency to clump.
Solution:	3.8% solution (single-strength), 7.6% (double strength), soluble in distilled or deionized water on boiling 2-3 minutes. Solution (single strength) is light amber, clear, may have a slight precipitate.
Prepared Medium:	Very light amber, clear, may have a very slight precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Prepare Vitamin B_{12} Assay Medium per label directions. Dispense into tubes with a titration from 0 to 0.25 ng of USP Cyanocobalamin Reference Standard. Inoculate with *L. delbrueckii* subsp. *lactis* ATCC® 4797 and incubate at 35-37°C for 18-24 hours. Turbidimetric measurements are taken using a spectrophotometer. The curve is then constructed from the values obtained.

Formula

Vitamin B_{12} 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	20 mg
Guanine	20 mg
Uracil	20 mg
Xanthine	1 mg
Thiamine Hydrochloride	2 mg
Riboflavin	2 mg
Niacin	2 mg
Calcium Pantothenate	200 µg
Pyridoxine Hydrochloride	4 mg
p-Aminobenzoic Acid	200 µg
Biotin	10 µg
Folic Acid	100 µg
Sorbitan Monooleate Complex	2 g
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 6.3 ± 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

Vitamin B_{12} Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus delbrueckii* subsp. *lactis* ATCC® 4797 or 7830
Sterile 0.85% saline
Distilled or deionized water
Centrifuge
0.1 N NaOH
Cyanocobalamin USP
Spectrophotometer or nephalometer
Lactobacilli Agar AOAC or B_{12} Culture Agar USP
Lactobacilli Broth AOAC or B_{12} Inoculum Broth USP

Method of Preparation

1. Suspend 7.6 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes.
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 assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Stock cultures of the test organism, *L. delbrueckii* subsp. *lactis* ATCC® 4797 or 7830, are prepared by stab inoculation of Lactobacilli Agar AOAC or B_{12} Culture Agar USP. Following incubation at 37°C for 24-48 hours, the tubes are stored in the refrigerator. Transfers are made at 2 week intervals.

Inoculum for the assay is prepared by subculturing a stock of *L. delbrueckii* subsp. *lactis* ATCC 4797 or 7830 into a tube containing 10 ml of Lactobacilli Broth AOAC or B_{12} Inoculum Broth USP. After incubation at 35-37°C for 18-24 hours, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are washed by resuspending in 10 ml of sterile 0.85% saline solution and centrifuging. The washing is repeated for a total of 3 times. Finally the cells are resuspended in 10 ml of sterile 0.85% saline. The cell suspension is then diluted 1:100 with sterile 0.85% saline. One drop is used to inoculate each assay tube.

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.

The concentrations required for the preparation of the standard curve are obtained by adding sufficient 25% ethanol to an accurately weighed amount of USP Cyanocobalamin Reference Standard (resulting in a solution containing 1.0 µg of cyanocobalamin per ml). This stock solution is stored in the refrigerator and should be used within 60 days.

In the preparation of the standard curve, further dilutions of this stock solution (1 µ/ml) are made as follows:

A. Add 1 ml stock solution to 99 ml distilled water (1 ml = 10 ng).
B. Add 1 ml of the solution from step A to 199 ml distilled water (1 ml = 0.05 ng).

An acceptable standard curve can be obtained by using the USP Cyanocobalamin Reference Standard at levels of 0.0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 ng per assay tube. This is accomplished by adding 0, 0.5, 1, 2, 3, 4 and 5 ml of the 0.05 ng/ml solution per assay tube and sufficient distilled or deionized water to make 10 ml volume per tube.

A standard concentration is used which, after incubation, gives a transmittance value at the 5 ml level of not less than that which corresponds to a dry cell weight of 1.25 mg (see USP[2] for method of calibration of a spectrophotometer and determination of dry cell weight). For the titrimetric method, a standard concentration should be used which, after incubation, will give a titration at the 5 ml level of 8-12 ml 0.1N sodium hydroxide.

Inoculate and incubate at 35-37°C for 18-24 hours. For turbidimetric determinations, place tubes in a refrigerator at 2-8°C for 15-20 minutes to stop growth. The growth can be measured by a nephelometric method. Titrimetric determinations of growth are made after incubation at 37°C for 72 hours. The curve is then constructed from the values obtained.

Results

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

Limitations of the Procedure

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

References

1. **Capps, Hobbs, and Fox.** 1949. J. Biol. Chem. **178**:517.
2. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.

Packaging

Vitamin B_{12} Assay Medium	100 g	0360-15*

*Store at 2-8°C

Bacto® WL Nutrient Medium · Bacto WL Nutrient Broth Bacto WL Differential Medium

Intended Use

Bacto WL Nutrient Medium and Bacto WL Nutrient Broth are used for cultivating yeasts, molds and bacteria encountered in brewing and industrial fermentation processes.

Bacto WL Differential Medium is used for isolating bacteria encountered in brewing and industrial fermentation processes.

Also Known As

WL Nutrient Medium is also referred to as "Wallerstein Laboratory Medium".

WL Nutrient Broth is also referred to as "Wallerstein Laboratory Nutrient Broth".

WL Differential Medium is also referred to as "Wallerstein Laboratory Differential Medium".

Summary and Explanation

WL Nutrient Media were developed by Green and Gray[1,2] in their study of various fermentation processes. An exhaustive study examining the methods of fermentation control procedures in worts, beers, liquid yeasts and similar fermentation products led to the development of WL Nutrient Media.

User Quality Control

Identity Specifications

WL Nutrient Medium and WL Differential Medium

Dehydrated

Media Appearance:	Light beige with a greenish tint, free-flowing, homogeneous.
Solution:	8.0% solution, soluble in distilled or deionized water on boiling. Solution is blue to greenish blue, slightly opalescent without significant precipitate.
Prepared Media:	Blue to greenish blue, slightly opalescent without significant precipitate.
Reaction of 8.0% Solution at 25° C:	pH 5.5 ± 0.2

WL Nutrient Broth

Dehydrated

Media Appearance:	Light beige with a greenish tint, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in distilled or deionized water. Solution is blue, clear without precipitate.
Prepared Medium:	Blue, clear without precipitation.
Reaction of 6.0% Solution at 25°C:	pH 5.5 ± 0.2

continued on following page

At a pH of 5.5, counts of viable bakers' yeast may be made on the WL Nutrient Medium. By adjusting the pH to 6.5, the medium is suitable for obtaining counts of bakers' and distiller's yeast. The medium can support the growth of bacteria, but unless the number of yeast cells is small the bacteria may not be detected. Due to this limitation, Green and Gray developed WL Differential Medium that inhibits the growth of yeasts without inhibiting the growth of bacteria present in beers.

WL Nutrient Agar and WL Differential Medium are used simultaneously as a set or three plates. One plate is prepared from WL Nutrient Agar and two plates from WL Differential Medium.[3] The WL Nutrient Agar plate is incubated aerobically to obtain a total count of mainly yeast colonies. A differential agar plate is incubated aerobically for growth of acetic acid bacteria, *Flavobacterium*, *Proteus* and thermophilic bacteria. Another differential agar plate is incubated anaerobically for growth of lactic acid bacteria and *Pediococcus*.

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins and amino acids. Casitone provides nitrogen, amino acids, and carbon. Dextrose is the source of carbohydrate. Monopotassium Phosphate buffers the media. Potassium Chloride, Calcium Chloride and Ferric Chloride are essential ions and help to maintain osmotic balance. Magnesium Sulfate and Manganese Sulfate are sources of divalent cations. Brom Cresol Green is a pH indicator.

Agar is the solidifying agent in WL Nutrient Medium and WL Differential Medium.

Actidione (cycloheximide) inhibits yeasts and molds in WL Differential Medium.

Formula

WL Nutrient Medium

Formula Per Liter

Bacto Yeast Extract	4 g
Bacto Casitone	5 g
Bacto Dextrose	50 g
Monopotassium Phosphate	0.55 g
Potassium Chloride	0.425 g
Calcium Chloride	0.125 g
Magnesium Sulfate	0.125 g
Ferric Chloride	0.0025 g
Manganese Sulfate	0.0025 g
Bacto Agar	20 g
Brom Cresol Green	0.022 g

Final pH 5.5 ± 0.2 at 25°C

WL Nutrient Broth

Formula Per Liter

Bacto Yeast Extract	4 g
Bacto Casitone	5 g
Bacto Dextrose	50 g
Monopotassium Phosphate	0.55 g
Potassium Chloride	0.425 g
Calcium Chloride	0.125 g
Magnesium Sulfate	0.125 g

Ferric Chloride ..... 0.0025 g
Manganese Sulfate ..... 0.0025 g
Brom Cresol Green ..... 0.022 g

Final pH 5.5 ± 0.2 at 25°C

WL Differential Medium

Formula Per Liter

Bacto Yeast Extract ..... 4 g
Bacto Casitone ..... 5 g
Bacto Dextrose ..... 50 g
Monopotassium Phosphate ..... 0.55 g
Potassium Chloride ..... 0.425 g
Calcium Chloride ..... 0.125 g
Magnesium Sulfate ..... 0.125 g
Ferric Chloride ..... 0.0025 g
Manganese Sulfate ..... 0.0025 g
Bacto Agar ..... 20 g
Brom Cresol Green ..... 0.022 g
Actidione® ..... 0.004 g

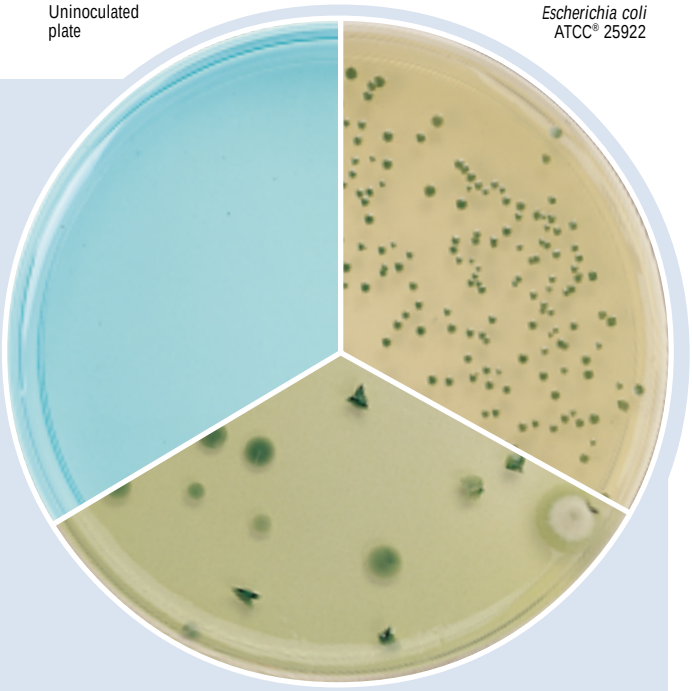
Final pH 5.5 ± 0.2 at 25°C

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.



User Quality Control cont.

Cultural Response

WL Nutrient Medium

Prepare WL Nutrient Medium per label directions. Inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	fair to good
Lactobacillus fermentum	9338	100-1,000	fair to good
Saccharomyces cerevisiae	9763	100-1,000	good

Escherichia coli ATCC® 25922 | *Lactobacillus fermentum* ATCC® 9338 | *Saccharomyces cerevisiae* ATCC® 9763

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.

WL Nutrient Broth

Prepare WL Nutrient Broth per label directions. Inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID	GAS
Escherichia coli	25922*	100-1,000	fair to good	+	+
Lactobacillus fermentum	9338	100-1,000	fair to good	+	sl. +
Saccharomyces cerevisiae	9763	100-1,000	good	+	+

Acid + = positive, yellow Acid – = negative, no color change

WL Differential Medium

Prepare WL Differential Medium per label directions. Inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Lactobacillus fermentum	9338	500-1,000	good
Saccharomyces cerevisiae	9763	1,000-2,000	inhibited

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

WL Nutrient Medium
WL Nutrient Broth
WL Differential Medium

Materials Required but not Provided

Glassware
Autoclave
Petri dishes
Tubes with closures
Fermentation tubes

Method of Preparation

WL Nutrient Medium and WL Differential Medium

1. Suspend 80 grams in 1 liter distilled or deionized water. OPTIONAL: To adjust the pH to 6.5, add the amount of 1% sodium carbonate solution specified on the product label to the rehydration water before dissolving the medium.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

WL Nutrient Broth

1. Dissolve 60 grams in 1 liter distilled or deionized water. OPTIONAL: Add fermentation tubes before sterilizing to assess gas production.
2. Dispense into tubes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Green, S. R., and P. P. Gray.** 1950. Paper read at American Society of Brewing Chemists Meeting. Wallerstein Lab. Commun. **12**:43.
2. **Green, S. R., and P. P. Gray.** 1950. A differential procedure applicable to bacteriological investigation in brewing. Wallerstein Lab. Commun. **13**:357.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 854-856. Williams & Wilkins, Baltimore, MD.

Packaging

WL Nutrient Medium	500 g	0424-17
	10 kg	0424-08
WL Nutrient Broth	500 g	0471-17
WL Differential Medium	500 g	0425-17

Bacto® XL Agar Base · Bacto XLD Agar

User Quality Control

Identity Specifications

XL Agar Base

Dehydrated Appearance:	Pink, homogeneous, free-flowing.
Solution:	4.7% solution, soluble in distilled or deionized water upon boiling. Solution is red, very slightly opalescent.
Prepared Plates:	Red, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 7.4 ± 0.2

XLD Agar

Dehydrated Appearance:	Pink, homogeneous, free-flowing.
Solution:	5.7% solution, soluble in distilled or deionized water upon boiling. Solution is red, very slightly opalescent.
Prepared Plates:	Red, slightly opalescent.
Reaction of 5.7% Solution at 25°C:	pH 7.4 ± 0.2

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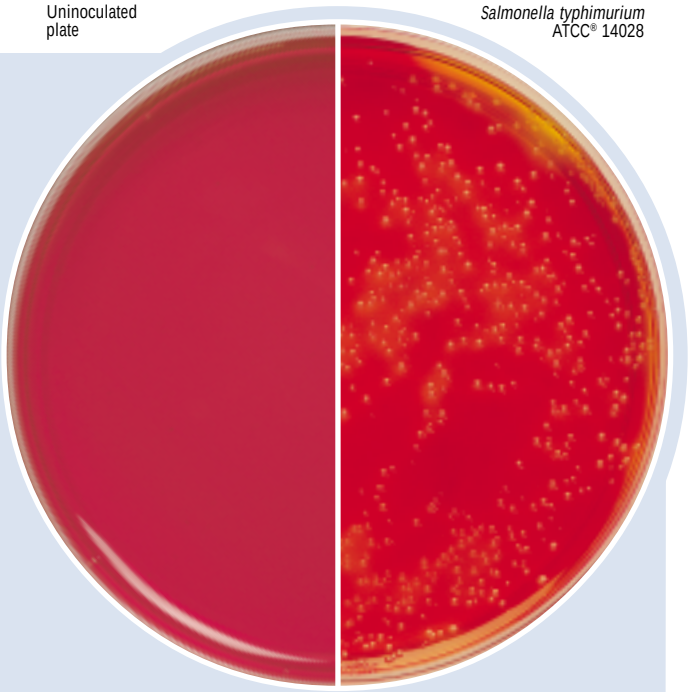


Intended Use

Bacto XL Agar Base is used with or without selective agents for isolating, differentiating and enumerating enteric bacteria.

Bacto XLD Agar is used for isolating and differentiating gram-negative enteric bacilli, especially *Shigella* and *Providencia*.

Also Known As

XL Agar with added brilliant green is referred to as XLBG Agar.

Summary And Explanation

XL (Xylose Lysine) Agar Base was developed by Taylor[1] for isolating and differentiating gram-negative enteric bacilli. The medium is nonselective, allowing growth of most enteric bacteria. XL Agar is recommended for performing counts of enteric organisms.

XL Agar Base can be supplemented with sodium thiosulfate and ferric ammonium citrate and, further, with sodium desoxycholate (2.5 grams per liter) to make XLD Agar. XL Agar Base can be made selective for *Salmonella* by adding brilliant green (1.25 ml of a 1% aqueous solution per liter) prior to autoclaving. The resulting XLBG Agar inhibits coliforms and *Shigella* and is recommended for isolating *Salmonella* following selenite or tetrathionate enrichment in food analysis.[1]

XLD Agar was developed principally for isolating *Shigella* and *Providencia*. It has been shown to be more effective than other enteric differential media.[2,3,4]

Principles of the Procedure

Yeast Extract provides sources of nitrogen and carbon, as well as vitamins and cofactors required for growth. Xylose, Lactose, and Sucrose (Saccharose) provide sources of fermentable carbohydrate. Xylose is fermented by most enteric organisms except *Shigella* and *Providencia*. Lysine is added to differentiate *Salmonella*. As xylose is exhausted, *Salmonella* organisms decarboxylate lysine causing a reversion to alkaline conditions. Alkaline reversion by other lysine-positive organisms is prevented by excess acid production from fermentation of lactose and sucrose.

Sodium Thiosulfate and Ferric Ammonium Citrate allow visualization of hydrogen sulfide production under alkaline conditions. Acidic conditions inhibit the reaction. Phenol Red is an indicator. Sodium Chloride maintains osmotic balance in the medium. Bacto Agar is a solidifying agent.

Sodium Desoxycholate in XLD Agar inhibits growth of gram-positive organisms.

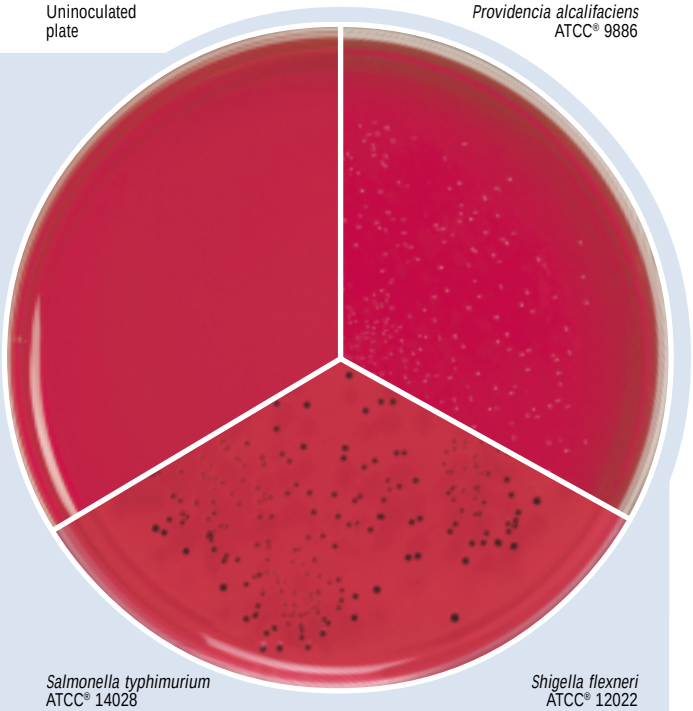
Formula

XL Agar Base

Formula Per Liter

Bacto Yeast Extract	3 g
L-Lysine	5 g
Bacto Xylose	3.75 g
Bacto Lactose	7.5 g
Bacto Saccharose	7.5 g
Sodium Chloride	5 g
Bacto Phenol Red	0.08 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C



User Quality Control cont.

Cultural Response

XL Agar Base

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	100-1,000	poor to fair	yellow
Escherichia coli	25922*	100-1,000	good	yellow
Salmonella typhimurium	14028*	100-1,000	good	red w/black centers
Shigella flexneri	12022*	100-1,000	good	red

XLD Agar

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1,000-2,000	partial inhibition	–
Escherichia coli	25922*	1,000-2,000	partial inhibition	yellow, may have a bile precipitate
Salmonella typhimurium	14028*	100-1,000	good	red w/black centers
Shigella flexneri	12022*	100-1,000	good	red

The organisms listed are the minimum used for performance testing.

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

XLD Agar

Formula Per Liter

Bacto Yeast Extract	3 g
L-Lysine	5 g
Bacto Xylose	3.75 g
Bacto Lactose	7.5 g
Bacto Saccharose	7.5 g
Sodium Desoxycholate	2.5 g
Ferric Ammonium Citrate	0.8 g
Sodium Thiosulfate	6.8 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Phenol Red	0.08 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. XLD Agar:

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

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

Storage

Store the dehydrated media 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

XL Agar Base
XLD Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Petri dishes
34% sodium thiosulfate, sterile (XL Agar)
4% ferric ammonium citrate, sterile (XL Agar)
1% brilliant green (XLBG)

Method of Preparation

XL Agar Base

1. Suspend 47 grams in 1 liter distilled or deionized water. (OPTIONAL: To prepare XLBG Agar, add 1.25 ml aqueous 1% solution of brilliant green.)
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
3. Cool to 55-60°C.
4. Aseptically add 20 ml sterile aqueous 34% solution of sodium thiosulfate and 4% solution of ferric ammonium citrate. Mix thoroughly.
5. Dispense as desired.

XLD Agar

1. Suspend 57 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Avoid overheating. **DO NOT AUTOCLAVE.**
3. Cool to 55-60°C. Dispense as desired.

Specimen Collection and Preparation

XLD Agar is listed in several procedures as a plating medium for the testing of food samples.[8,9,10,11] Refer to the appropriate references regarding specimen preparation.

Test Procedure

Feces or rectal swabs may be plated directly; selective enrichment broths, such as Selenite Broth or Tetrathionate Broth, may be used prior to streaking.[5,6,7]

Results

Degradation of xylose, lactose and sucrose generates acid products, causing a color change in the medium from red to yellow.

Hydrogen sulfide production under alkaline conditions causes colonies to develop black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.

Lysine decarboxylation in the absence of lactose and sucrose fermentation causes reversion to an alkaline condition and the color of the medium changes back to red.

Limitations of the Procedure

1. Red, false-positive colonies may occur with some *Proteus* and *Pseudomonas* species.
2. Incubation in excess of 48 hours may lead to false-positive results.
3. *S. paratyphi A, S. choleraesuis, S. pullorum* and *S. gallinarum* may form red colonies without black centers, thus resembling *Shigella* species.
4. Some *Proteus* strains will give black-centered colonies on XLD Agar.

References

1. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars; new media for isolation of enteric pathogens. Am. J. Clin. Pathol. **44**(4):471-475.
2. **Rollender, W., O. Beckford, R. D. Belsky, and B. Kostroff.** 1969. Comparison of xylose lysine deoxycholate agar and MacConkey agar for the isolation of *Salmonella* and *Shigella* from clinical specimens. Tech. Bull. Reg. Med. Tech. **39**(1):8-10.
3. **Pollock, H. M., and B. J. Dahlgren.** 1974. Clinical evaluation of enteric media in the primary isolation of *Salmonella* and *Shigella*. Appl. Microbiol. **27**(1):197-201.

4. **Bhat, P., and D. Rajan.** 1975. Comparative evaluation of desoxycholate citrate medium and xylose lysine desoxycholate medium in the isolation of shigellae. Am. J. Clin. Pathol. **64**:399-404.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
8. **Smith, J. L., and R. L. Buchanan.** 1992. *Shigella*, p. 423-431. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
9. **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.
10. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. *In* Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.
11. **Association of Official Analytical Chemists.** 1996. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.

Packaging

XL Agar Base	500 g	0555-17
XLD Agar	100 g	0788-15
	500 g	0788-17
	2 kg	0788-07
	10 kg	0788-08

Bacto® XLT4 Agar Base
Bacto XLT4 Agar Supplement

Intended Use

Bacto XLT4 Agar Base is used with Bacto XLT4 Agar Supplement in isolating non-*typhi Salmonella*.

User Quality Control

Identity Specifications

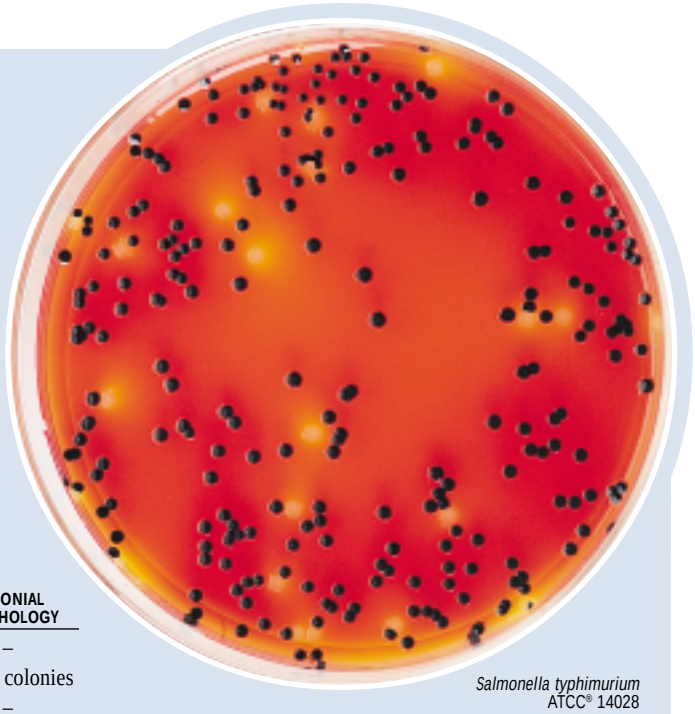
Dehydrated Appearance:	Pink, free flowing, homogeneous.
Base Solution:	5.9% solution; soluble in distilled or deionized water on boiling. Solution is red, very slightly to slightly opalescent.
XLT4 Agar Supplement:	Colorless to slightly yellow, clear, slightly viscous solution.
Prepared Medium:	Reddish-orange, very slightly to slightly opalescent.
Reaction of Final Medium at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare XLT4 Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	CFU	INOCULUM GROWTH	COLONIAL MORPHOLOGY
Enterococcus faecalis	29212*	1,000	markedly inhibited	–
Escherichia coli	25922*	1,000	partially inhibited	yellow colonies
Proteus mirabilis	25933	1,000	inhibited	–
Salmonella typhimurium	14028*	100-1,000	good	yellow to red colonies with black centers
Staphylococcus aureus	25923*	1,000	inhibited	–

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



Salmonella typhimurium ATCC® 14028 on XLT4 Agar Base with XLT4 Supplement

Summary and Explanation

Numerous media have been developed for isolating and differentiating enteric pathogens. The majority were designed to recover a broad spectrum of enteric pathogens.[1] Consequently, overgrowth of nuisance or contaminating organisms can be a major problem when recovery of a specific organism or species is desired. This is particularly true for *Salmonella* isolation media where overgrowth of *Proteus*, *Providencia* and *Pseudomonas* can dramatically interfere with the detection and isolation of *Salmonella*.

In 1990, Miller and Tate described a new medium, XLT4 Agar, for isolating *Salmonella*.[1] The authors established the selectivity of XLT4 Agar using pure cultures of a variety of enteric organisms. They also evaluated its sensitivity in detecting and isolating *Salmonella* using fecal-contaminated farm samples containing high numbers of competing bacteria. In follow-up studies, Miller[2,3] and Tate[4] reported that XLT4 Agar significantly improved the recovery of non-*typhi Salmonella* from chicken and farm environmental drag-swab samples.

XLT4 Agar can be used clinically to screen stool samples for non-typhoid *Salmonella*.[5,6]

Principles of the Procedure

XLT4 Agar Base contains Proteose Peptone No. 3 as a source of complex nitrogen compounds. Yeast Extract is added as a source of vitamins and other cofactors. Differentiation of *Salmonella* from other organisms that also grow on this medium is based on fermentation of Xylose, Lactose and Sucrose, decarboxylation of Lysine, and the production of hydrogen sulfide. Hydrogen sulfide production is detected by the addition of ferric ions. Sodium Thiosulfate is added as a source of inorganic sulfur. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent. Phenol Red is added as an indicator of pH changes resulting from fermentation and decarboxylation reactions. XLT4 Agar Supplement is added to inhibit growth of non-*Salmonella* organisms.

Formula

XLT4 Agar Base

Formula Per Liter

Bacto Proteose Peptone No. 3	1.6 g
Bacto Yeast Extract	3 g
L-Lysine	5 g
Bacto Xylose	3.75 g
Bacto Lactose	7.5 g
Bacto Saccharose	7.5 g
Ferric Ammonium Citrate	0.8 g
Sodium Thiosulfate	6.8 g
Sodium Chloride	5 g
Bacto Agar	18 g
Bacto Phenol Red	0.08 g

Final pH 7.4 ± 0.2 at 25°C

XLT4 Agar Supplement

A 27% solution (approximate) of the surfactant 7-ethyl-2-methyl-4-undecanol hydrogen sulfate, sodium salt, formerly produced by Union Carbide under the tradename of Tergitol 4.

Precautions

1. For Laboratory Use.
2. **XLT4 Agar Base**

   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.

   XLT4 Agar Supplement

   CORROSIVE. CAUSES BURNS. HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing, gloves and eye/face protection. 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 XLT4 Agar Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Store XLT4 Agar Supplement 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

XLT4 Agar Base
XLT4 Agar Supplement

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 59 grams of XLT4 Agar Base in 1 liter distilled or deionized water.
2. Add 4.6 ml XLT4 Agar Supplement.
3. Heat to boiling to dissolve completely. Avoid overheating. **DO NOT AUTOCLAVE**. Cool to 45-50°C in a waterbath.
4. 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.[7-10]

2. Process each specimen, using procedures appropriate for that sample.[7,8,10,11]

Test Procedure

1. Inoculate a suitable *Salmonella* enrichment broth (such as Tetrathionate Broth) and incubate at 35°C for 18-24 hours.
2. Following enrichment, subculture onto XLT4 Agar. Streak for isolation.
3. Incubate plates aerobically at 35 ± 2°C. Examine for growth after 18-24 and 48 hours incubation.

Results

Typical *Salmonella* colonies (H_2S-positive) appear black or black-centered with a yellow periphery after 18-24 hours of incubation. Upon continued incubation, the colonies become entirely black or pink to red with black centers.

Colonies of H_2S-negative *Salmonella* strains appear pinkish-yellow.

Most *Citrobacter* colonies that grow on this medium are yellow without evidence of blackening. Growth of *Enterobacter aerogenes* and *Escherichia coli* is markedly inhibited; colonies that do grow appear yellow without evidence of blackening. Growth of *Proteus*, *Pseudomonas*, *Providencia*, *Alteromonas putrefaciens*, *Yersinia enterocolitica* and *Acinetobacter calcoaceticus* is markedly to completely inhibited on XLT4 Agar. *Shigella* species are partially inhibited and colonies appear red.

Limitations of the Procedure

1. XLT4 Agar is intended for detecting and isolating *Salmonella* based on selectivity and colonial characteristics. Presumed *Salmonella* colonies must be confirmed by biochemical and/or immunological methods. Consult appropriate references for further information.[5,7,8,12]
2. Since the nutritional requirements of organisms vary, some strains of *Salmonella* may be encountered that fail to grow or grow poorly on this medium.
3. Non-*Salmonella* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Salmonella*. Consult appropriate references.[7,8,10,12]
4. Freshly inoculated plates and plates held over several days may develop multicolored, metallic looking crystals/flecks on the surface. These crystals/flecks do not interfere with the performance of the medium.

References

1. **Miller, R. G., and C. R. Tate**. 1990. XLT4: A highly selective plating medium for the isolation of *Salmonella*. The Maryland Poultryman April:2-7.
2. **Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Schemer**. 1991. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Science **70**:2429-2432.
3. **Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Schemer**. 1992. *Erratum*. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Science **71**:398.
4. **Tate, C. R., R. G. Miller, and E. T. Mallinson**. 1992. Evaluation of two isolation and two non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag-swab samples. J. Food Prot. **55**:964-967.
5. **Dusch, H., and M. Altwegg**. 1994. Evaluation of Xylose-Lysine-Tergitol 4 (XLT4) Agar and Modified Semisolid Rappaport Vassiliadis (MSRV) Medium for the isolation of non-typhoid salmonellae from stool samples. Abstr. Annu. Meet. Am. Soc. Microbiol. **C5**:557.
6. **Dusch, H., and M. Altwegg**. 1995. Evaluation of five new plating media for the isolation of *Salmonella* species. J. Clin. Microbiol. **33**:802-804.
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8. **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.
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12. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*. *In* P. R. Murray, *et al.* (ed), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

XLT4 Agar Base	500 g	0234-17
XLT4 Agar Supplement	l00 ml	0353-72

Bacto® YM Agar
Bacto YM Broth

Intended Use

Bacto YM Agar and YM Broth are used for cultivating yeasts, molds and other aciduric microorganisms.

Also Known As

YM is an abbreviation for Yeast Extract and Malt Extract.

Summary and Explanation

YM Agar and YM Broth are prepared according to the formulation published by Wickerham.[1,2,3] Wickerham suggested that YM Broth acidified to pH 3.0-4.0 be used as an enrichment medium for yeasts from populations also containing bacteria and molds. To favor isolation of fermentative species, add a layer of sterile paraffin oil

1 cm deep on the surface of the inoculated broth. Incubate the culture until growth appears and then streak onto YM Agar to obtain isolated yeast colonies. To isolate fermentative and oxidative strains, place acidified inoculated YM Broth on a rotary shaker for 1 or 2 days. This favors yeast recovery while preventing the sporulation of molds.

Media selectivity may be enhanced through acidification or through addition of selective agents. YM Broth may be acidified prior to sterilization. YM Agar should be sterilized without pH adjustment and sterile acid added to the sterile molten medium cooled to 45-50°C. Acidified YM Agar should not be heated. Antibiotics may be aseptically added to the sterile media. Other fungistatic materials, such as sodium propionate and diphenyl may be added to YM Agar to eliminate molds and permit the enumeration of yeasts to mixed populations.

User Quality Control

Identity Specifications

YM Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly opalescent, without significant precipitate.
Prepared Plates:	Light to medium amber, slightly opalescent without precipitate.
Reaction of 4.1% Solution at 25°C:	pH 6.2 ± 0.2

YM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear to very slightly opalescent without significant precipitate.
NOTE:	At pH adjusted to 3.0-4.0, medium becomes slightly opalescent.
Prepared Tubes:	Light - medium amber, clear to very slightly opalescent without significant precipitate.
Reaction of 2.1% Solution at 25°C:	pH 6.2 ± 0.2

Cultural Response

Prepare 2 sets of YM Agar plates or YM Broth tubes (one set pH 6.2, one set adjusted to pH 3.0-4.0) per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH PH 3.0-4.0	GROWTH PH 6.2
Aspergillus niger	16404	100-1,000	good	good
Candida albicans	10231	100-1,000	good	good
Escherichia coli	25922*	100-1,000	markedly to completely inhibited	good
Lactobacillus casei	7469	100-1,000	poor to fair	good
Saccharomyces cerevisiae	9763	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.

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins and amino acids. Malt Extract is a source of carbon, protein and nutrients. Bacto Peptone is an additional source of carbon and provides nitrogen and amino acids. Dextrose provides carbon. Bacto Agar is a solidifying agent.

Formula

YM 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

Final pH 6.2 ± 0.2 at 25°C

YM Broth

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Bacto Peptone	5 g
Bacto Dextrose	10 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

YM Agar
YM Broth

Materials Required but not Provided

Glassware
Autoclave
Antibiotics
Sterile 10% HCl, Tartaric Acid or 10% Citric Acid

Method of Preparation

YM Agar

1. Suspend 41 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.

YM Broth

1. Dissolve 21 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

   ***Optional* (for Agar or Broth):** If desired, acidify the medium to pH 3.0-4.0 by adding sterile 10% HCl, Tartaric Acid or 10% Citric Acid. Selective agents, e.g., penicillin (20 units per ml final concentration) or streptomycin (40 micrograms per ml final concentration) may be added to the medium after sterilization using aseptic technique.

Test Procedure

1. Inoculate YM Agar plates or YM Broth tubes with sample to evaluated for the presence of yeasts, molds, or aciduric microorganisms.
2. Incubate at 30 ± 2°C for 18-72 hours.

Results

Examine the plates or tubes for growth. Record YM Agar results as colony forming units (CFU) per volume of sample. Record YM Broth results as growth or no growth.

Limitations of the Procedure

1. Acidified YM Agar should not be overheated.

References

1. 1951. U. S. Dept. Agricult. Tech. Bull. No. 1029.
2. 1939. J. Tropical Med. Hyg. **42:**176.
3. **Jong, S. C., and M. J. Edwards**. 1991. American Type Culture Collection Catalog of filamentous fungi, 18th ed. American Type Collection, Rockville, MD.

Packaging

YM Agar	500 g	0712-17
YM Broth	500 g	0711-17
	10 kg	0711-08

Bacto® YPD Agar
Bacto YPD Broth

User Quality Control

Identity Specifications

YPD Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 6.5 ± 0.2

YPD Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Medium:	Light to medium amber, clear to very slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

Prepare YPD Agar or YPD Broth per label directions. Inoculate and incubate the plates or tubes at 25 ± 2°C for 42-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Kluyveromyces lactis	8563	100-1,000	good
Saccharomyces cerevisiae	18790	100-1,000	good
Saccharomyces pastorianus	9080	100-1,000	good

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

Intended Use

Bacto YPD Agar and Bacto YPD Broth are used for maintaining and propagating yeasts in molecular microbiology procedures.

Also Known As

YPD media are also known as Yeast Extract-Peptone-Glucose media and may be abbreviated as YEPD.

Summary and Explanation

General methods in yeast genetics specify using yeast extract-peptone-glucose (YPD) medium for cultivating *Saccharomyces cerevisiae* and other yeasts.[1] Yeasts grow well on a minimal medium containing only glucose and salts. The addition of protein and yeast cell extract hydrolysates allow faster growth so that during exponential or log-phase growth, the cells divide every 90 minutes.[1]

Principles of the Procedure

YPD Agar and YPD Broth contain Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. YPD Agar contains Bacto Agar as the solidifying agent.

Formula

YPD Agar

Formula Per Liter

Bacto Yeast Extract	10 g
Bacto Peptone	20 g
Bacto Dextrose	20 g
Bacto Agar	15 g

Final pH 6.5 ± 0.2 at 25°C

YPD Broth

Formula Per Liter

Bacto Yeast Extract	10 g
Bacto Peptone	20 g
Bacto Dextrose	20 g

Final pH 6.5 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

YPD Agar
YPD Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (25°C)

Method of Preparation

YPD Agar

1. Suspend 65 grams of YPD Agar in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

YPD Broth

1. Suspend 50 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

See appropriate references for specific procedures.

Results

Growth of colonies on the agar or in the broth (turbidity).

References

1. **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., Current Protocols, Brooklyn, NY.

Packaging

YPD Agar	500 g	0427-17
	2 kg	0427-07
YPD Broth	500 g	0428-17
	2 kg	0428-07

Yeast Extract

Bacto® Yeast Extract · Bacto Yeast Extract, Technical Bacto Autolyzed Yeast

Intended Use

Bacto Yeast Extract, Bacto Yeast Extract, Technical and Bacto Autolyzed Yeast are used in preparing microbiological culture media.

Summary and Explanation

Yeast Extract is the water soluble portion of autolyzed yeast. The autolysis is carefully controlled to preserve the naturally occurring B-complex vitamins. Yeast Extract is prepared and standardized for bacteriological use. It is an excellent stimulator of bacterial growth and used in culture media in place of, or in addition to, beef extract. Yeast Extract is generally employed in the concentration of 0.3-0.5%.

Yeast Extract has been used successfully in culture media for studies of bacteria in milk and other dairy products. The advantage of Yeast Extract for this purpose is documented by Prickett[1] on the thermophilic and thermoduric bacteria of milk. Since publication of Prickett's[1] study, Yeast Extract has been used more frequently in the study of bacterial flora in milk. Hutner[2] used this product in a stock broth for streptococci. Partansky and McPherson[3] used Yeast Extract in combination with Bacto Malt Extract and Bacto Agar for testing mold resistant properties of oil paints.

Yeast Extract is an excellent source of B-complex vitamins and is often used to supply these factors in bacteriological culture media. Snell and Strong[4] used Yeast Extract for the preparation of yeast supplement in their medium for riboflavin assay. It has been a valuable ingredient for carrying stock cultures, and for preparation of inocula of lactobacilli for microbiological assay of vitamins. This product is also of value in the assay of antibiotics. A growth substance, B factor for *Norcardia*, can be isolated from Yeast Extract.[5] Yeast Extract supplies this factor necessary for a rifampin mutant to product rifampin.[5]

Several media containing Yeast Extract are specified in standard methods for multiple applications.[6,7,8,9]

Yeast Extract, Technical is a water soluble portion of autolyzed yeast containing vitamin B complex. Yeast Extract, Technical is used in bacterial culture media when a standardized yeast extract is not essential. It demonstrates acceptable clarity and growth promoting characteristics.

Autolyzed Yeast is a desiccated product containing both the soluble and insoluble portions of autolyzed bakers' yeast. It is recommended for preparation of yeast supplements used in microbiological assay for riboflavin and pantothenic acid.[10,11]

Principles of the Procedure

Yeast Extract is typically prepared by growing baker's yeast, *Saccharomyces* sp., in a carbohydrate-rich plant medium. The yeast is harvested, washed and resuspended in water, where is undergoes autolysis, i.e., self digestion using yeast's enzymes. Yeast Extract is the total soluble portion of this autolytic action. The autolytic activity is stopped by a heating step. The resulting Yeast Extract is then filtered clear and subsequently made a powder by the spray drying process.

Yeast Extract, Yeast Extract, Technical and Autolyzed Yeast provide vitamins, nitrogen, amino acids and carbon in microbiological culture media.

User Quality Control

Identity Specifications

Yeast Extract

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1% solution - soluble in distilled or deionized water. Solution is light to medium amber, clear, may have a very slight precipitate. 2% solution-medium amber, clear, may have a very slight precipitate.
Reaction of 1% Solution at 25°C:	pH 6.6 ± 0.2

Yeast Extract, Technical

Dehydrated Appearance:	Light to medium beige, free-flowing, homogeneous.
Solution:	1% solution - soluble in distilled or deionized water. Solution is light to medium amber in color, clear to very slightly opalescent, may have a precipitate.

Autolyzed Yeast

Dehydrated Appearance:	Medium to dark brown, homogenous, free-flowing.
Solution:	1% solution - not completely soluble in distilled or deionized water upon boiling. Solution is amber, opaque, may have a precipitate.
Reaction of 1% Solution at 25°C:	pH 4.9-6.3

Cultural Response

Yeast Extract

Prepare a solution containing 1% Yeast Extract and 0.5% sodium chloride. Adjust the pH to 7.2 ± 0.2 using dilute NaOH. Inoculate tubes with the test organisms and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	fair to good
Staphylococcus aureus	25923*	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good

Yeast Extract, Technical

Prepare a solution containing 2% Yeast Extract with 0.5% sodium chloride. Adjust the pH to 7.2 ± 0.2 using dilute NaOH. Inoculate tubes with the test organisms and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	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.

Typical Analysis

Yeast Extract

Physical Characteristics

Ash (%)	11.2	Loss on Drying (%)	3.1
Clarity, 1% Soln (NTU)	1.5	pH, 1% Soln	6.7
Filterability (g/cm2)	2.7		

Carbohydrate (%)

Total	17.5

Nitrogen Content (%)

Total Nitrogen	10.9	AN/TN	55.0
Amino Nitrogen	6.0		

Amino Acids (%)

Alanine	5.36	Lysine	5.15
Arginine	3.02	Methionine	1.05
Aspartic Acid	6.69	Phenylalanine	2.53
Cystine	0.74	Proline	2.60
Glutamic Acid	14.20	Serine	2.84
Glycine	3.25	Threonine	2.95
Histidine	1.20	Tryptophan	1.36
Isoleucine	3.23	Tyrosine	1.20
Leucine	4.69	Valine	3.79

Inorganics (%)

Calcium	0.013	Phosphate	3.270
Chloride	0.380	Potassium	3.195
Cobalt	<0.001	Sodium	1.490
Copper	<0.001	Sulfate	0.091
Iron	<0.001	Sulfur	0.634
Lead	<0.001	Tin	<0.001
Magnesium	0.075	Zinc	0.011
Manganese	<0.001		

Vitamins (µg/g)

Biotin	3.3	PABA	763.0
Choline (as Choline Chloride)	300.0	Pantothenic Acid	273.7
Cyanocobalamin	<0.1	Pyridoxine	43.2
Folic Acid	1.5	Riboflavin	116.5
Inositol	1400.0	Thiamine	529.9
Nicotinic Acid	597.9	Thymidine	17.5

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	60
Salmonella	negative	Thermophile Count	<5
Spore Count	9		

Precautions

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

Storage
Store the dehydrated ingredient below 30°C. The 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
Yeast Extract
Yeast Extract, Technical
Autolyzed Yeast

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

Method of Preparation
Refer to the final concentration of Yeast Extract, Yeast Extract, Technical or Autolyzed Yeast in the formula of the medium being prepared. Add Yeast Extract, Yeast Extract, Technical or Autolyzed Yeast 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 Yeast Extract, Yeast Extract, Technical or Autolyzed Yeast.

Results
Refer to appropriate references and procedures for results.

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

References

1. **Prickett.** 1928. Tech. Bull. 147. NY Agr. Exp. Sta.
2. **Hutner.** 1938. J. Bacteriol. **35**:429.
3. **Partansky and McPherson.** 1940. Ind. Eng. Chem., Anal. Ed. **12**:443.
4. **Snell and Strong.** 1939. Ind. Eng. Chem., Anal. Ed. **11**:346.
5. **Kawaguchi, T., T. Asahi, T. Satoh, T. Uozumi, and T. Beppu.** 1984. B-factor, an essential regulatory substance inducing the production of rifamycin in a *Nocardia* sp. J. Antibiot. **37**:1587-1594.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
8. **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.
9. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
10. **J. Ind. Eng. Chem., Anal. Ed.** 1941. **13**:567.
11. **J. Ind. Eng. Chem., Anal. Ed.** 1942. **14**:909.

Packaging
Yeast Extract	100 g	0127-15
	500 g	0127-17
	2 kg	0127-07
	10 kg	0127-08
Yeast Extract, Technical	500 g	0886-17
	10 kg	0886-08
Autolyzed Yeast	500 g	0229-17
	10 kg	0229-08

Bacto® Yeast Extract Glucose Chloramphenicol Agar

Intended Use
Bacto Yeast Extract Glucose Chloramphenicol Agar is a selective agar recommended by the International Dairy Federation[1,2] for enumerating yeasts and molds in milk and milk products.

Also Known As
Yeast Extract Glucose Chloramphenicol Agar is also known as YGC Agar.

Summary and Explanation
The antibiotic method for enumerating yeasts and molds in dairy products has become the method of choice, replacing the traditional acidified method.[2] The use of antibiotics for suppressing bacteria results in better recovery of injured fungal cells, which are sensitive to an acid environment, and in less interference from precipitated food particles during the counting.[3-7]

Yeast Extract Glucose Chloramphenicol Agar is a nutrient medium that inhibits the growth of organisms other than yeasts and molds due to the presence of Chloramphenicol. When a sample contains predominantly yeasts and/or injured yeasts, the use of Yeast Extract Glucose Chloramphenicol Agar may offer some advantage.[2] After incubation at 25°C, colonies are counted and yeast colonies are distinguished from molds by colony morphology.

Principles of the Procedure
Yeast Extract provides basic nutrients. Glucose is a carbon energy source. Chloramphenicol inhibits bacterial growth.

Formula
Yeast Extract Glucose Chloramphenicol Agar

Formula per liter

Bacto Yeast Extract	5 g
Glucose	20 g
Chloramphenicol	0.1 g
Bacto Agar	13 g

Final pH 6.6 ± 0.2 at 25°C

Precautions

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

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

Storage

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

Yeast Extract Glucose Chloramphenicol Agar
Materials Required but not Provided
Glassware
Distilled or deionized water
Autoclave
Petri dishes
Ringer's solution
2% Dipotassium phosphate
2% Sodium citrate

Method of Preparation

1. Suspend 38.1 grams in 1 liter distilled or deionized water.
2. Boil gently to dissolve completely.
3. Dispense 10-12 ml aliquots into tubes or other final containers and cap loosely.
4. Autoclave at 121°C for 15 minutes.

Test Procedure

1. Prepare initial sample dilutions using 10 grams or 10 ml of sample in 90 ml of diluent, as listed below:

SAMPLE 10 grams or 10 ml	DILUENT 90 ml	PREPARATION
Milk Liquid milk product	1/4-strength Ringer's solution	Mix.
Dried Milk Whey powder Buttermilk powder Lactose	1/4-strength Ringer's solution	Shake at 47°C.
Casein	2% dipotassium phosphate solution	Shake at 47°C.
Cheese	2% sodium citrate solution	Shake at 47°C.
Butter Edible ice	1/4-strength Ringer's solution	Shake at 47°C.
Custard dessert Fermented milk Yogurt	1/4-strength Ringer's solution	Shake.

2. Add 10 ml from the initial dilution prepared above (#1) to 90 ml of 1/4-strength Ringer's solution. One milliliter (1 ml) of this dilution corresponds to 0.01 gram/ml of sample.
3. Prepare further dilutions by adding 10 ml of the 0.01 gram/ml dilution above (#2) to 90 ml of diluent.
4. Pipette 1 ml of each dilution into two Petri dishes.
5. Pour 10 ml of sterile molten agar (cooled to 45°C) into each dish. Mix thoroughly.
6. Incubate at 25°C for 4 days.

Results

1. Select plates containing 10-300 colonies and count the colonies. Distinguish yeasts from molds by colony morphology.
2. Express results as yeasts and molds "per gram" or "per milliliter."

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.81% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear to slightly opalescent.
Reaction of 3.81% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Prepare the medium per label directions. Inoculate by the pour plate technique and incubate at 25 ± 2°C for up to 4 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	30-300	good
Candida albicans	10231	30-300	good
Escherichia coli	25922*	1,000-2,000	inhibited
Saccharomyces cerevisiae	9763	30-300	good

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

References

1. **International Dairy Federation.** Standard Method ISO/DIS 6611.
2. **Frank, J. F., G. L. Christen, and L. B. Bullerman.** 1993. Tests for groups of microorganisms, p. 281-283. *In* R. T. Marshall, (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Beuchat, L. R.** 1979. Comparison of acidified and antibiotic-supplemented potato dextrose agar from three manufacturers for its capacity to recover fungi from foods. J. Food Prot. **42**:427-428.
4. **Cooke, W. B., and A. R. Brazis.** 1968. Occurrence of molds and yeasts in dairy products. Mycopathol. Mycol. Appl. **35**:281-289.

5. **Koburger, J. A.** 1970. Fungi in foods: 1. Effect of inhibitor and incubation temperature on enumeration. J. Milk Food Technol. **33**:433-434.
6. **Koburger, J. A.** 1973. Fungi in foods: 5. Response of natural populations to incubation temperatures between 12 and 32°C. J. Milk Food Technol. **36**:434- 435.
7. **Overcase, W. W., and D. J. Weakley.** 1969. An aureomycin-rose bengal agar for enumeration of yeast and mold in cottage cheese. J. Milk Food Technol. **32**:442- 445.

Packaging

Yeast Extract Glucose Chloramphenicol Agar	500 g	1900-17
	5 kg	1900-03

Yeast Media

Bacto® Yeast Morphology Agar · Bacto Yeast Carbon Base Bacto Yeast Nitrogen Base · Bacto Yeast Nitrogen Base w/o Amino Acids · Bacto Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

User Quality Control

Identity Specifications

Yeast Morphology Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is very light amber, very slightly to slightly opalescent.
Prepared Medium:	Very light amber, slightly opalescent without significant precipitate.
Reaction of 3.5% Solution at 25°C:	pH 5.6 ± 0.2

Yeast Carbon Base

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	1.17% (single-strength) and 11.7% (10X) solution, soluble in distilled or deionized water with slight warming. Single-strength solution is colorless to very light amber, clear after filter-sterilization.
Prepared Medium:	Colorless to very light amber, clear, no precipitate.
Reaction of 1.17% Solution at 25°C:	pH 5.5 ± 0.2

Yeast Nitrogen Base

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	0.67% (single strength) and 6.7% (10X) solution, soluble in distilled or deionized water with agitation. Single-strength solution is almost colorless and clear; 10X solution is yellow and clear.
Prepared Medium:	Colorless, clear without precipitate.
Reaction of 0.67% Solution at 25°C:	pH 5.4 ± 0.2

continued on following page

Intended Use

Bacto Yeast Morphology Agar is used for classifying yeasts based on colonial characteristics and cell morphology.

Bacto Yeast Carbon Base is used for classifying yeasts based on nitrogen assimilation.

Bacto Yeast Nitrogen Base is used for classifying yeasts based on carbon assimilation.

Bacto Yeast Nitrogen Base w/o Amino Acids is used for classifying yeasts based on amino acid and carbohydrate requirements.

Bacto Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate is used for classifying yeasts based on carbon and nitrogen requirements.

Summary and Explanation

Yeasts are unicellular, eukaryotic, budding cells that are generally round-to-oval or elongate in shape.[1] They multiply principally by the production of blastoconidia (buds).[1] Yeast colonies are moist and creamy or glabrous to membranous in texture.[1] Yeasts are considered opportunistic pathogens.[1]

The yeast media cited are prepared according to the formulas of Wickerham.[2,3,4,5,6]

Yeast Carbon Base tests the ability of yeasts to assimilate nitrogen by the addition of various nitrogen sources. The inclusion of vitamins aids in the utilization of nitrogen-containing compounds by certain yeasts which cannot assimilate these compounds in the absence of vitamins.

Yeast Nitrogen Base is a suitable medium for studying strains of yeast that require certain vitamins.

Yeast Nitrogen Base w/o Amino Acids, which lacks the amino acids histidine, methionine and tryptophane, and Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate, which lacks amino acids and ammonium sulfate, are prepared according to Guenter's[7] modification of Wickerham's Yeast Nitrogen Base formulation.

These media are included in many applications for the study of yeasts in molecular genetics.[8,9]

Principles of the Procedure

Yeast Morphology Agar contains all essential nutrients and vitamins necessary for the cultivation of yeasts, including a source of carbohydrate.

Yeast Carbon Base contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of nitrogen.

Yeast Nitrogen Base contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of carbohydrate.

Yeast Nitrogen Base w/o Amino Acids contains all essential vitamins and inorganic salts necessary for the cultivation of yeasts except histidine, methionine, tryptophane and a source of carbohydrate.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate contains all essential nutrients and vitamins necessary for the cultivation of yeasts except amino acids and a source of nitrogen and carbohydrate.

Formula

Yeast Morphology Agar

Formula per Liter

Nitrogen Sources

Ammonium Sulfate	3.5 g
Asparagine	1.5 g

Carbon Source

Dextrose	10 g

Amino Acids

L-Histidine Monohydrochloride	10 mg
LD-Methionine	20 mg
LD-Tryptophan	20 mg

Vitamins

Biotin	2 µg
Calcium Pantothenate	400 µg
Folic Acid	2 µg
Inositol	2,000 µg
Niacin	400 µg
p-Aminobenzoic Acid	200 µg
Pyridoxine Hydrochloride	400 µg
Riboflavin	200 µg
Thiamine Hydrochloride	400 µg

User Quality Control cont.

Yeast Nitrogen Base w/o Amino Acids

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	0.67% (single strength) or 6.7% (10X) solution, soluble in distilled or deionized water with agitation. Single-strength solution is colorless to very pale yellow and clear; 10X solution is yellow and clear.
Prepared Medium:	Colorless, clear without precipitate.
Reaction of 0.67% Solution at 25°C:	pH 5.4 ± 0.2

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Dehydrated Appearance:	Light yellowish-beige, free-flowing, homogeneous.
Solution:	0.17% (single-strength) and 1.7% (10X) solution, soluble in distilled or deionized water. Single-strength solution is colorless to very pale yellow and clear; 10X solution is yellow and clear.
Prepared Medium:	Colorless, clear without precipitate.
Reaction of 0.17% Solution at 25°C:	pH 4.5 ± 0.2

Cultural Response

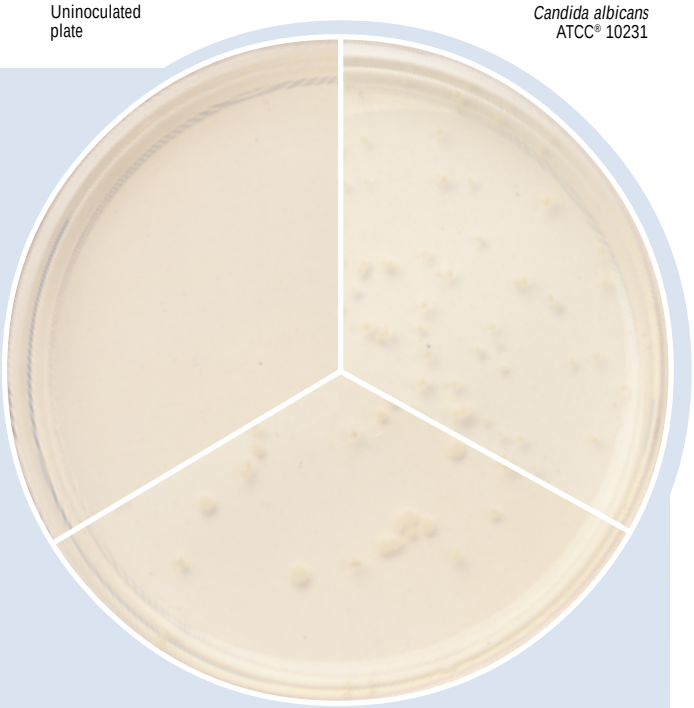
Yeast Morphology Agar

Prepare Yeast Morphology Agar per label directions. Inoculate using the pour plate technique and incubate at 25-30°C for 18-48 hours. Also, inoculate by the Dolman technique (streak and point) and incubate at 25-30°C for 6-7 days.

ORGANISM	ATCC®	GROWTH	DOLMAN PLATE TEST
Kloeckera apiculata	9774	good	–
Saccharomyces pastorianus	9080	good	–
Candida albicans	10231	good	hyphae

Uninoculated plate

Candida albicans ATCC® 10231



Saccharomyces pastorianus ATCC® 9080

Yeast Carbon Base (with and without 5% ammonium sulfate)
Yeast Nitrogen Base (with and without 5% dextrose)
Yeast Nitrogen Base w/o Amino Acids (with and without 5% dextrose, 0.02% DL-methionine, 0.02% DL-tryptophane and 0.015% L-histidine)
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate (with and without 5% dextrose, 5% ammonium sulfate, 0.02% DL-methionine, 0.02% DL-tryptophane and 0.01% L-histidine)

Prepare the medium per label directions with and without the supplements indicated above. Inoculate and incubate at 25-30°C for 2-5 days.

ORGANISM	ATCC®	GROWTH WITHOUT SUPPLEMENT(S)	GROWTH WITH SUPPLEMENT(S)
Kloeckera apiculata	9774	none to poor	good
Saccharomyces pastorianus	9080	none to poor	good

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

Compounds Supplying Trace Elements
Boric Acid ..... 500 µg
Copper Sulfate ..... 40 µg
Potassium Iodide ..... 100 µg
Ferric Chloride ..... 200 µg
Manganese Sulfate ..... 400 µg
Sodium Molybdate ..... 200 µg
Zinc Sulfate ..... 400 µg

Salts
Potassium Phosphate Monobasic ..... 1 g
Magnesium Sulfate ..... 0.5 g
Sodium Chloride ..... 0.1 g
Calcium Chloride ..... 0.1 g
Bacto Agar ..... 18 g

Final pH 5.6 ± 0.2 at 25°C

Yeast Carbon Base

Formula per Liter

Carbon Source
Dextrose ..... 10 g

Amino Acids
L-Histidine Monohydrochloride ..... 1 mg
LD-Methionine ..... 2 mg
LD-Tryptophan ..... 2 mg

Vitamins
Biotin ..... 2 µg
Calcium Pantothenate ..... 400 µg
Folic Acid ..... 2 µg
Inositol ..... 2,000 µg
Niacin ..... 400 µg
p-Aminobenzoic Acid ..... 200 µg
Pyridoxine Hydrochloride ..... 400 µg
Riboflavin ..... 200 µg
Thiamine Hydrochloride ..... 400 µg

Compounds Supplying Trace Elements
Boric Acid ..... 500 µg
Copper Sulfate ..... 40 µg
Potassium Iodide ..... 100 µg
Ferric Chloride ..... 200 µg
Manganese Sulfate ..... 400 µg
Sodium Molybdate ..... 200 µg
Zinc Sulfate ..... 400 µg

Salts
Potassium Phosphate Monobasic ..... 1 g
Magnesium Sulfate ..... 0.5 g
Sodium Chloride ..... 0.1 g
Calcium Chloride ..... 0.1 g

Final pH 5.5 ± 0.2 at 25°C

Yeast Nitrogen Base

Formula per Liter

Nitrogen Sources
Ammonium Sulfate ..... 5 g

Amino Acids
L-Histidine Monohydrochloride ..... 10 mg
LD-Methionine ..... 20 mg
LD-Tryptophan ..... 20 mg

Vitamins
Biotin ..... 2 µg
Calcium Pantothenate ..... 400 µg
Folic Acid ..... 2 µg
Inositol ..... 2,000 µg
Niacin ..... 400 µg
p-Aminobenzoic Acid ..... 200 µg
Pyridoxine Hydrochloride ..... 400 µg
Riboflavin ..... 200 µg
Thiamine Hydrochloride ..... 400 µg

Compounds Supplying Trace Elements
Boric Acid ..... 500 µg
Copper Sulfate ..... 40 µg
Potassium Iodide ..... 100 µg
Ferric Chloride ..... 200 µg
Manganese Sulfate ..... 400 µg
Sodium Molybdate ..... 200 µg
Zinc Sulfate ..... 400 µg

Salts
Potassium Phosphate Monobasic ..... 1 g
Magnesium Sulfate ..... 0.5 g
Sodium Chloride ..... 0.1 g
Calcium Chloride ..... 0.1 g

Final pH 5.4 ± 0.2 at 25°C

Yeast Nitrogen Base w/o Amino Acids

Formula per Liter

Nitrogen Sources
Ammonium Sulfate ..... 5 g

Vitamins
Biotin ..... 2 µg
Calcium Pantothenate ..... 400 µg
Folic Acid ..... 2 µg
Inositol ..... 2,000 µg
Niacin ..... 400 µg
p-Aminobenzoic Acid ..... 200 µg
Pyridoxine Hydrochloride ..... 400 µg
Riboflavin ..... 200 µg
Thiamine Hydrochloride ..... 400 µg

Compounds Supplying Trace Elements
Boric Acid ..... 500 µg
Copper Sulfate ..... 40 µg
Potassium Iodide ..... 100 µg
Ferric Chloride ..... 200 µg
Manganese Sulfate ..... 400 µg
Sodium Molybdate ..... 200 µg
Zinc Sulfate ..... 400 µg

Salts
Potassium Phosphate Monobasic ..... 1 g
Magnesium Sulfate ..... 0.5 g
Sodium Chloride ..... 0.1 g
Calcium Chloride ..... 0.1 g

Final pH 5.4 ± 0.2 at 25°C

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Formula per Liter

Vitamins
Biotin ..... 2 µg
Calcium Pantothenate ..... 400 µg
Folic Acid ..... 2 µg
Inositol ..... 2,000 µg
Niacin ..... 400 µg
p-Aminobenzoic Acid ..... 200 µg

Pyridoxine Hydrochloride	400 µg
Riboflavin	200 µg
Thiamine Hydrochloride	400 µg

Compounds Supplying Trace Elements

Boric Acid	500 µg
Copper Sulfate	40 µg
Potassium Iodide	100 µg
Ferric Chloride	200 µg
Manganese Sulfate	400 µg
Sodium Molybdate	200 µg
Zinc Sulfate	400 µg

Salts

Potassium Phosphate Monobasic	1 g
Magnesium Sulfate	0.5 g
Sodium Chloride	0.1 g
Calcium Chloride	0.1 g

Final pH 4.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Yeast Morphology 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, Intestines.

   Yeast Nitrogen Base

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

   Yeast Nitrogen Base w/o Amino Acids

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL IF SWALLOWED. (EC) 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

Yeast Morphology Agar
Yeast Carbon Base
Yeast Nitrogen Base
Yeast Nitrogen Base w/o Amino Acids
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Materials Required But Not Provided

Glassware
Filter sterilization equipment
Sterile distilled or deionized water
Sterile test tubes
Spectrophotometer
Petri dishes
Pipettes
Dextrose or an equivalent carbohydrate (Yeast Nitrogen Base)
Nitrogen source (Yeast Carbon Base)

Method of Preparation

Yeast Morphology Agar

1. Suspend 35 grams of Yeast Morphology Agar in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Pour the sterile medium into plates to a depth of approximately 1.5 mm.
5. Allow the plates to stand at room temperature.

Yeast Carbon Base

1. Prepare a 10X solution by dissolving 11.7 grams of Yeast Carbon Base and a nitrogen source in 100 ml distilled or deionized water.

   NOTE: When using potassium nitrate, an important nitrogen-containing compound in nitrogen assimilation testing, add 0.78 grams.
2. Warm to dissolve, if necessary. Mix well.
3. Filter-sterilize the solution.
4. Store at 2-8°C.
5. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution into 4.5 ml of sterile distilled water.
6. Mix the solution thoroughly by shaking before inoculation.

Yeast Nitrogen Base

1. Prepare a 10X solution by dissolving 6.7 grams of Yeast Nitrogen Base and 5 grams of Dextrose or an equivalent amount of other carbohydrate in 100 ml distilled or deionized water.
2. Warm slightly to dissolve. Mix well.
3. Filter-sterilize the solution.
4. Store at 2-8°C.
5. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution into 4.5 ml of sterile distilled water.
6. Mix the solution thoroughly by shaking before inoculation.

Yeast Nitrogen Base w/o Amino Acids

1. Prepare a 10X solution by dissolving 6.7 grams of Yeast Nitrogen Base w/o Amino Acids and 5 grams of Dextrose or an equivalent amount of other carbohydrate and 5-10 mg% of the desired amino acid in 100 ml of distilled or deionized water.
2. Filter sterilize the solution.
3. Store at 2-8°C.
4. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution into 4.5 ml of sterile distilled water.
5. Mix the solution thoroughly by shaking before inoculation.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

1. Prepare a 10X solution by suspending 1.7 grams of Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate and nitrogen and carbon sources, as required, in 100 ml distilled or deionized water.
2. Filter sterilize the solution.
3. Store at 2-8°C.
4. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution into 4.5 ml sterile distilled water.
5. Mix the solution thoroughly by shaking before inoculation.

Specimen Collection and Preparation

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

Procedure

Yeast Morphology Agar

Inoculate plates using the Dolman technique (as follows) described by Wickerham and Rettger.[1] This is an excellent method for studying the hyphae of filamentous yeasts.

1. Near one side of the plate (from the relative positions of 10 o'clock to 2 o'clock), lightly inoculate a single streak taken from a slant culture.
2. In addition to the single streak, inoculate two points near the other side of the plate (at the 4 o'clock and 8 o'clock positions).
3. Cover a central section of the streak inoculation and one point inoculation with cover glasses, as follows:
   a. With forceps, remove a cover glass from absolute alcohol, drain momentarily, and burn off excess alcohol by passing over a low flame.
   b When the cover glass has cooled, place one edge on the agar and allow it to fall across the central portion of the inoculated streak. Place a second cover glass over one point inoculation.
4. Incubate at 25-30°C for 6-7 days.
5. After incubation, observe with a high dry objective.

Yeast Carbon Base, Yeast Nitrogen Base, Yeast Nitrogen Base w/o Amino Acids, Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

1. Inoculate the prepared tubed medium very lightly with the test organism.
2. Incubate at 25°C for 6-7 days.
3. After incubation (6-7 days and, if necessary, 20-24 days), shake the tubes to suspend growth.
4. Read for growth.

Carbon Assimilation Test

Refer to the procedure described in the Manual of Clinical Microbiology.[10]

Nitrogen Assimilation Test

Refer to the procedure described in the Manual of Clinical Microbiology.[10]

Results

Yeast Morphology Agar

Using the high-dry objective, observe for hyphae of filamentous yeasts.

Yeast Carbon Base, Yeast Nitrogen Base, Yeast Nitrogen Base w/o Amino Acids, Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Measure growth turbidimetrically at 660 nm wavelength using a spectrophotometer. Turbidimetric readings on assay tubes should be comparable to the control.

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 a medium.
2. Yeasts grown on a rich medium may carry a reserve of nitrogen in the form of protein. Possible errors due to this reserve are eliminated by making two serial transfers in the complete medium. When the first transfer is seven days old, the culture is shaken and one loopful is transferred to a second tube of the complete medium containing the same source of nitrogen. If a positive test is obtained when the second culture is seven days old, the organism being tested assimilates this particular nitrogen source.

References

1. **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.
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3. **Wickerham, L. J.** 1939. J. Tropical Med. Hyg. **42**:176.
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5. **Wickerham, L. J.** 1948. J. Bacteriol. **56**:363.
6. **Wickerham, L. J.** 1943. J. Bacteriol. **46**:501.
7. **Guenter.** Personal Communication.
8. **Sherman F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
9. **Brownstein, B. H., G. A. Silverman, R. D. Little, D. T. Burke, S. J. Korsmeyer, D. Schlessinger, and M. V. Olson.** 1989. Isolation of single-copy human genes from a library of yeast artificial chromosomes clones. Science. **244**:1348-1351.
10. **Warren, N. G., and H. J. Shadomy.** 1991. Yeasts of medical importance, p. 617- 629. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.). Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Packaging

Yeast Morphology Agar	100 g	0393-15
Yeast Carbon Base	100 g	0391-15
Yeast Nitrogen Base	100 g	0392-15
Yeast Nitrogen Base w/o Amino Acids	100 g	0919-15
	2 kg	0919-07
	10 kg	0919-08
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate	100 g	0335-15
	10 kg	0335-08

Yersinia Selective Agar

Bacto® Yersinia Selective Agar Base · Bacto Yersinia Antimicrobic Supplement CN

Intended Use

Bacto Yersinia Selective Agar Base is used with Bacto Yersinia Antimicrobic Supplement CN in isolating and cultivating *Yersinia enterocolitica*.

Also Known As

Yersinia Selective Agar is also known as CIN Agar, Modified or Cefsulodin-Irgasan-Novobiocin Agar, Modified.

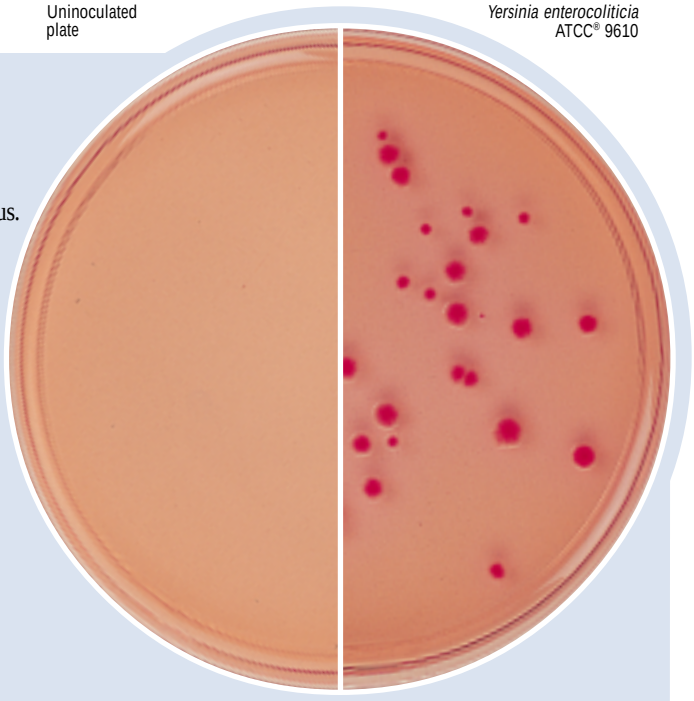
Summary and Explanation

Yersinia enterocolitica is a significant enteric pathogen[6] and can be food- or water- borne.[7]

Yersinia Selective Agar is a selective and differential medium that supports good growth of *Y. enterocolitica* and some other *Yersinia* species. The formulation is based on the Cefsulodin-Irgasan-Novobiocin (CIN) Agar formulation of Schiemann.[2-5] In comparison with MacConkey Agar and Salmonella-Shigella Agar, Schiemann found that CIN Agar provided better inhibition of normal enteric organisms and provided improved direct recovery of *Y. enterocolitica* from feces.[3] Schiemann later modified his original formula by substituting 0.5 grams of deoxycholate for the bile salts mixture and by reducing the content of novobiocin to 2.5 mg/liter for improved growth of strains of *Y. enterocolitica* serogroup 0:8.[5] The concentration of cefsulodin in the antimicrobic supplement was reduced from that described by Schiemann to further improve growth and recovery of *Y. enterocolitica*.

Principles of the Procedure

Selectivity of Yersinia Selective Agar Base is due to the presence of bile salts, crystal violet and Irgasan®, which markedly inhibit growth of gram-positive and many gram-negative organisms. Supplementation with Yersinia Antimicrobic Supplement CN (Cefsulodin and Novobiocin) improves inhibition of normal enteric organisms. Differentiation is based on mannitol fermentation. Organisms capable of fermenting



User Quality Control

Identity Specifications

Yersinia Selective Agar Base

Dehydrated Appearance: Light pinkish beige, free-flowing, homogeneous.

Solution: 5.95% solution, soluble in distilled or deionized water upon boiling. Reddish purple, very slightly to slightly opalescent.

Prepared Medium: Reddish orange, very slightly to slightly opalescent.

Reaction of a 5.95% Solution at 25°C: pH 7.4 ± 0.2

Yersinia Antimicrobic Supplement CN

Dehydrated Appearance: Lyophilized, white, homogeneous cake.

Solution: Soluble on rehydration with distilled or deionized water, colorless, clear.

Reaction of Solution at 25°C: pH 5.2-6.3

Cultural Response

Prepare Yersinia Selective Agar according to label directions. Inoculate and incubate at 30 ± 2°C for 18-24 hours or at 22-25°C for 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	2,000-10,000	marked to complete inhibition	
Escherichia coli	25922*	2,000-10,000	marked to complete inhibition	
Pseudomonas aeruginosa	27853*	2,000-10,000	marked to complete inhibition	
Yersinia enterocolitica	9610	100-1,000	good	colorless with dark pink centers; bile precipitate may be present

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.

mannitol produce a pH decrease around the colony which allows absorption of neutral red, giving the colony a red color. Due to the localized pH decrease, a zone of precipitated bile may also be present. Organisms that do not metabolize mannitol to acid end products will form colorless, translucent colonies.

Formula

Yersinia Selective Agar Base

Formula Per Liter	
Bacto Yeast Extract	2 g
Bacto Peptone	17 g
Bacto Proteose Peptone	3 g
Mannitol	20 g
Sodium Deoxycholate	0.5 g
Sodium Cholate	0.5 g
Sodium Chloride	1 g
Sodium Pyruvate	2 g
Magnesium Sulfate Heptahydrate	10 mg
Bacto Agar	13.5 g
Neutral Red	30 mg
Crystal Violet	1 mg
Irgasan	4 mg

Yersinia Antimicrobic Supplement CN

Formula Per 10 ml Vial	
Cefsulodin	4 mg
Novobiocin	2.5 mg

Precautions

1. For Laboratory Use.
2. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Liver, Blood.

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

Storage

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

Store Yersinia Antimicrobic Supplement CN lyophilized and rehydrated at 2-8°C. Do not open or rehydrate vials until ready to use. Use the rehydrated product 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

Yersinia Selective Agar Base
Yersinia Antimicrobic Supplement CN

Materials Required But Not Provided

Glassware
Distilled or deionized water
Incubator (22-25°C or 30 ± 2°C)
Autoclave
Sterile Petri dishes

Method of Preparation

Yersinia Antimicrobic Supplement CN

1. To rehydrate the supplement, aseptically add 10 ml sterile distilled or deionized water to the vial.
2. Invert the vial gently several times to dissolve the powder.

Yersinia Selective Agar Base

1. Suspend 59.5 grams in 1 liter distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes. Avoid overheating.
3. Cool the medium to 45-50°C. Aseptically add 10 ml rehydrated Yersinia Antimicrobic Supplement CN to the medium. Mix well.
4. Dispense into Petri dishes.

Specimen Collection and Preparation

All specimens should be collected in sterile containers in accordance with recommended guidelines and should be transported immediately to the laboratory. For specific information on collection and storage of specimens consult appropriate references.

Test Procedure

For a complete discussion on the isolation and identification of *Yersinia*, consult appropriate references.

Results

Y. enterocolitica colonies appear translucent or translucent with dark pink centers. Colony edges are entire or irregular. After 48 hours incubation, colonies appear dark pink with a translucent border and may be surrounded by a zone of precipitated bile.

Growth of non-*Yersinia* organisms is markedly to completely inhibited.

Limitations of the Procedure

1. Yersinia Selective Agar Base and Yersinia Antimicrobic Supplement CN are intended for use in the preparation of Yersinia Selective Agar. Although this medium is selective for *Yersinia*, biochemical testing using pure cultures is necessary for complete identification.
2. Due to the selective properties of the medium, some *Yersinia* strains may be encountered that fail to grow or grow poorly on the complete medium. Some strains of normal enteric organisms may be encountered that are not inhibited or are only partially inhibited on the complete medium, such as *Citrobacter freundii*, *Serratia liquefaciens* and *Enterobacter agglomerans*.
3. Growth of *Yersinia frederiksenii*, *Y. kristensenii*, *Y. pseudotuberculosis* and *Y. intermedia* is not inhibited on the complete medium. Colonies of these organisms must be differentiated from *Y. enterocolitica* on the basis of additional characteristics.

References

1. **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.

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6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Yersinia Selective Agar Base	500	g	1817-17
	10	kg	1817-08
Yersinia Antimicrobic Supplement CN	6 x 10	ml	3196-60