PALCAM Medium

Bacto® PALCAM Medium Base · Bacto PALCAM Antimicrobic Supplement

Intended Use

Bacto PALCAM Medium Base is used with Bacto PALCAM Antimicrobic Supplement in isolating and cultivating *Listeria* from foods.

Summary and Explanation

PALCAM Medium Base and PALCAM Antimicrobic Supplement are based on the PALCAM agar formulation of van Netten et al.,[1] who developed this selective and differential medium for use in the isolation and enumeration of *Listeria* spp. from food samples. PALCAM medium is recommended by AFNOR for use in the detection of *L. monocytogenes* in foods,[2] and by the IDF as an additional plating medium for the detection of *Listeria* spp. in milk and milk products.[3] PALCAM medium is recommended by Health Canada for the detection of *L. monocytogenes* in food and environmental samples.[4]

Principles of the Procedure

Good growth of *Listeria* spp. is obtained by including Columbia Blood Agar Base in PALCAM Medium Base. Columbia Blood Agar Base provides the nutrients and cofactors required for good to excellent growth of *Listeria*. Selectivity of the complete medium is achieved through the presence of Lithium Chloride, Polymyxin B Sulfate and Acriflavine HCl, present in PALCAM Medium Base, and Ceftazidime, provided by PALCAM Antimicrobic Supplement. These agents effectively suppress growth of most commonly occurring non-*Listeria* spp. of bacteria present in foods. The ceftazidime concentration is reduced from 20 mg/l to 8 mg/l for improved growth and recovery of *Listeria*.

Differentiation on PALCAM Medium is based on esculin hydrolysis and mannitol fermentation. All *Listeria* spp. hydrolyze esculin as evidenced by a blackening of the medium. This blackening by esculin-hydrolyzing bacteria results from the formation of 6,7 dihydroxycoumarin, which reacts with ferric ions that are present in the medium as Ferric Ammonium Citrate. On occasion, organisms other than *Listeria*, such as staphylococci or enterococci, may grow on this medium. Mannitol and the pH indicator, Phenol Red, have been added to differentiate mannitol-fermenting strains of these species from *Listeria* based on mannitol fermentation. Mannitol fermentation is demonstrated by a color change in the colony and/or the surrounding medium from red or gray to yellow due to the production of acidic end products.

User Quality Control

Identity Specifications

PALCAM Medium Base

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 6.8% solution, soluble in distilled or deionized water on boiling; dark red, very slightly to slightly opalescent with a slight precipitate.

Reaction of 6.8 % Solution at 25°C: pH 7.2 ± 0.2

PALCAM Antimicrobic Supplement

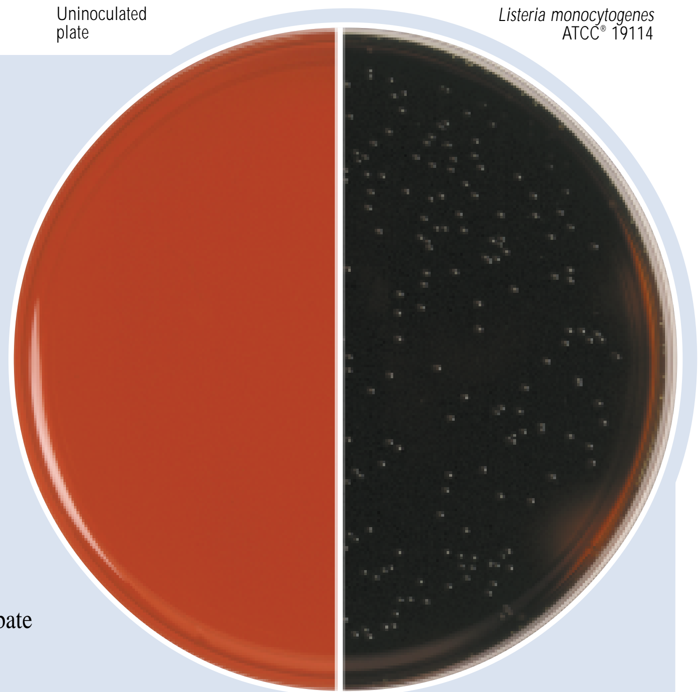
Lyophilized Appearance: White, free-flowing, homogeneous powder.

Rehydrated Appearance: Colorless solution.

Cultural Response

Prepare PALCAM Medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours under microaerophilic conditions.

ORGANISM	ATCC®	INOCULUM	GROWTH
Escherichia coli	25922*	1,000-2,000	inhibited
Listeria monocytogenes	19114	100-1,000	good growth, gray-green colonies with black precipitate
Staphylococcus aureus	25923*	1,000-2,000	inhibited
Enterococcus faecalis	29212*	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.

Formula

PALCAM Medium Base

Formula Per Liter

Bacto Columbia Blood Agar Base	39 g
Bacto Mannitol	10 g
Bacto Dextrose	0.5 g
Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Lithium Chloride	15 g
Phenol Red	0.08 g
Acriflavine HCl	0.005 g
Polymyxin B Sulfate	0.01 g
Bacto Agar	2 g

Final pH 7.2 ± 0.2 at 25°C

PALCAM Antimicrobic Supplement

Formula per 10 ml vial

Ceftazidime	40 mg

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.
3. PALCAM Medium Base:

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET 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.
4. PALCAM Antimicrobic Supplement:

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

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

Storage

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

Store PALCAM Antimicrobic Supplement at 2-8°C.

Store the rehydrated supplement and 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

PALCAM Medium Base
PALCAM Antimicrobic Supplement

Materials Required But Not Provided

Flasks with closures
Sterile distilled or deionized water
Autoclave
Waterbath (45-50°C)
Incubator (35°C)

Depending on testing method:

Fraser Broth Base
Demi-Fraser Broth Base
Fraser Broth Supplement
Oxford Medium Base
Oxford Antimicrobic Supplement
Modified Oxford Antimicrobic Supplement
Listeria Enrichment Broth
Modified Listeria Enrichment Broth
Tryptic Soy Agar with 0.6% Yeast Extract
LPM Agar Base
Moxalactam Antimicrobic Supplement

Method of Preparation

1. Suspend 68 grams PALCAM Medium Base in 1 liter distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
3. Aseptically add 2 ml PALCAM Antimicrobic Supplement which has been rehydrated with 10 ml sterile distilled or deionized water. Mix well.

Specimen Collection and Preparation

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

Test Procedure

A number of methods and incubation conditions may be used for detecting and isolating *Listeria* on PALCAM Medium. In their original work, van Netten et al. recommended incubation at 37°C for 48 hours under microaerophilic conditions.[1] AFNOR, HPB and IDF methods for detecting *Listeria* in foods and dairy products are listed below. Consult guidelines appropriate to your country and sample type.

AFNOR Method for Foods[2]

1. Pre-enrich the sample in Demi-Fraser Broth. Incubate at 30°C for 18-24 hours. Subculture onto Oxford Medium or PALCAM Medium.
2. Transfer 0.1 ml of the pre-enrichment culture into 10 ml of Fraser Broth and incubate at 37°C for 48 hours. Subculture onto Oxford Medium or PALCAM Medium after 18-24 and 42-48 hours of incubation.
3. After the required incubation, examine for presumptive *Listeria* colonies.

4. Confirm the identity of each presumptive *Listeria* isolate by biochemical and/or serological testing.

IDF Method for Milk and Milk Products[3]

1. Enrich the sample in Modified Listeria Enrichment Broth. Incubate at 30 ± 1°C for 48 ± 2 hours.
2. Subculture onto Oxford Medium (and onto PALCAM Medium, if desired). Incubate at 37 ± 1°C for 48 ± 2 hours.
3. After the required incubation, examine for presumptive *Listeria* colonies.
4. Subculture five presumptive colonies (or all of the colonies if there are less than five) from each isolation medium onto Tryptic Soy Agar with 0.6 % Yeast Extract.
5. Confirm the identity of each presumptive *Listeria* isolate by biochemical and/or serological testing.

Health Canada Method for Foods and Environmental Samples[4]

1. Enrich the sample in Listeria Enrichment Broth (LEB). Incubate at 30°C for 48 hours.
2. Transfer 0.1 ml of the primary enrichment broth culture into 9.9 ml of modified Fraser Broth. Incubate at 35°C for 24-48 hours. (If desired, the LEB culture may also be streaked onto Oxford Medium [OXA] and lithium chloride-phenylethanol-moxalactam agar [LPM], modified Oxford medium [MOX] or PALCAM medium [PAL]. Incubate LPM at 30°C for 24-48 hours and OXA, MOX and PAL at 35°C for 24-48 hours.)
3. Examine modified Fraser broth for reactions. Subculture all positive cultures (black, dark brown or dark green) after 24 and 48 hours of incubation onto OXA and LPM, MOX or PAL, streaking for isolation. Incubate LPM at 30°C for 24-48 hours and OXA, MOX and PAL at 35°C for 24-48 hours. If desired, all negative modified Fraser broth cultures (straw color) may be subcultured onto OXA and LPM, MOX or PAL to facilitate recovery of esculin-negative strains of *L. monocytogenes*.
4. Examine for presumptive *Listeria* colonies. Examine LPM under oblique lighting positioned at a 45° angle relative to the surface of the plate.
5. Confirm the identity of each presumptive *Listeria* isolate by biochemical and/or serological testing.

Results

On PALCAM Medium, colonies of *Listeria* appear gray-green with a black precipitate following inoculation and incubation at 35°C for 24-48 hours under aerobic or microaerophilic conditions. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/serological identification.[2,3] Colonies of mannitol-fermenting organisms such as staphylococci, which may grow on this medium, appear yellow with a yellow halo.

References

1. **Van Netten, P., I. Perales, A. Van de Moosalijk, G. D. W. Curtis, and D. A. A. Mossel.** 1989. Liquid and solid selective differential media for the detection and enumeration of *L. monocytogenes* and other *Listeria* spp. Int. J. of Food Microbiol. **8:**299-317.
2. **L'association française de normalisation (AFNOR).** 1993. Food Microbiology- Detection of *Listeria monocytogenes*-Routine Method, V 08-055. AFNOR, Paris.
3. **International Dairy Federation.** 1990. Milk and milk products-Detection of *Listeria monocytogenes*. IDF Provisional International Standard no. 143. International Dairy Federation, Brussels.
4. **Farber, J. M., D. W. Warburton, and T. Babiuk.** 1994. Isolation of *Listeria monocytogenes* from all food and environmental samples. Health Protection Branch Ottawa, MFHPB-30. Polyscience Publications, Quebec.

Packaging

PALCAM Medium Base	500 g	0636-17
	2 kg	0636-07
PALCAM Antimicrobic Supplement	3 x 10 ml	0637-57*

*Store at 2-8°C

Bacto® PKU Test Agar · Bacto PKU Test Agar w/o Thienylalanine Bacto Subtilis Spore Suspension No. 2

Intended Use

Bacto PKU Test Agar is used with Bacto Subtilis Spore Suspension No. 2 in estimating the phenylalanine level in blood.

Bacto PKU Test Agar w/o Thienylalanine is used with Bacto Subtilis Spore Suspension No. 2 and ß-2-thienylalanine in estimating phenylalanine levels in blood.

Also Known As

The Guthrie Modified Bacterial Inhibition Assay (BIA) for PKU

Phenylketonuria (PKU) results from an inborn error of phenylalanine metabolism. In this disease, phenylalanine hydroxylase deficiency causes accumulation of the amino acid phenylalanine with subsequent neurological damage.

In 1934, Folling[1] reported the presence of a urine phenylalanine metabolite in mentally retarded persons. Jervis[2] established that defective phenylalanine metabolism was the cause of the mental retardation. Detection and management of PKU are possible by testing infants for abnormal levels of phenylalanine or its metabolites. The Guthrie bacterial inhibition assay (BIA), which estimates the level of phenylalanine in the blood, is used for this purpose.[3,4,5,6] *Bacillus subtilis* ATCC® 6633 growth is inhibited in minimal culture medium containing ß-2-thienylalanine. Phenylalanine blocks the inhibition, allowing the organism to grow.

In the PKU Test procedure, PKU Test Agar containing thienylalanine or PKU Test Agar w/o Thienylalanine with added thienylalanine are inoculated with a suspension of *B. subtilis* ATCC® 6633. Filter paper disks saturated with infant blood and control disks impregnated with known concentrations of L-phenylalanine (2,4,6,8,12 and 20 mg%) are applied to the surface of the medium. After incubation at 35°C for 12-16 hours, the zones of growth around the test disks are compared to the zones around the control disks. A growth zone around the test disk comparable to the zone around the 4 mg% or higher disk is a presumptive positive indication of phenylketonuria. A positive result must be repeated using a duplicate test disk and a chemical or spectrofluorometric procedure.[7,8]

Principles of the Procedure

PKU Test Agar and PKU Test Agar w/o Thienylalanine are defined minimal media containing the factors necessary for *B. subtilis* growth under appropriate conditions. ß-2-thienylalanine is an inhibitor of *B. subtilis* growth. PKU Test Agar contains the inhibitor, ß-2-thienylalanine; PKU Test Agar w/o Thienylalanine does not, requiring the user to add ß-2-thienylalanine to the medium. Phenylalanine supplied from a PKU-positive patient specimen will overcome the inhibitory action of ß-2-thienylalanine.

Formula

PKU Test Agar

Formula Per Liter

L-Glutamic Acid	0.5 g
DL-Alanine	0.5 g
Bacto Asparagine	0.5 g
Bacto Dextrose	10 g
Dipotassium Phosphate	15 g
Monopotassium Phosphate	5 g
Ammonium Chloride	2.5 g
Ammonium Nitrate	0.5 g
Sodium Sulfate	0.5 g
Magnesium Sulfate	0.05 g
Manganese Chloride	0.005 g
Ferric Chloride	0.005 g
Calcium Chloride	0.0025 g
B_2 Thienylalanine	0.0033 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

PKU Test Agar w/o Thienylalanine

Formula Per Liter

L-Glutamic Acid	0.5 g
DL-Alanine	0.5 g
Bacto Asparagine	0.5 g
Bacto Dextrose	10 g
Dipotassium Phosphate	15 g
Monopotassium Phosphate	5 g
Ammonium Chloride	2.5 g
Ammonium Nitrate	0.5 g
Sodium Sulfate	0.5 g
Magnesium Sulfate	0.05 g
Manganese Chloride	0.005 g
Ferric Chloride	0.005 g
Calcium Chloride	0.0025 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Bacillus Spore Suspension No. 2

Standardized, stable suspension of *Bacillus subtilis* ATCC® 6633 containing 1.2 to 1.8 x 10^8 spores/ml.

User Quality Control

Identity Specifications

PKU Test Agar, PKU Test Agar w/o Thienylalanine

Dehydrated Appearance:	Light beige to beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent with a slight precipitate.
Prepared Medium:	Light amber, very slightly to slightly opalescent with a slight precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.0 ± 0.2

Subtilis Spore Suspension No. 2

Appearance:	White, opalescent, homogeneous suspension.

Cultural Response

PKU Test Agar, PKU Test Agar w/o Thienylalanine

Prepare the final medium per label directions. Apply PKU Standard Disks. Incubate at 35 + 2°C for 12-16 hours. Measure zones of growth around each PKU Standard Disk. Zones of growth should increase in size comparable to the increasing concentration of phenylalanine in the Standard Disks.

Precautions

1. For Laboratory Use.
2. **PKU Test Agar**
   PKU Test Agar w/o Thienylalanine
   HARMFUL. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Skin, 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. **Bacillus Spore Suspension No. 2**
   CAUTION. While spore suspensions are not considered to be pathogens, they are, nevertheless, live organisms. Never use mouth pipetting. Always use some type of pipetting aid.
4. 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 Subtilis Spore Suspension No. 2 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

PKU Test Agar
PKU Test Agar w/o Thienylalanine
Subtilis Spore Suspension No. 2

Materials Required but not Provided

ß-2-thienylalanine (use with PKU Test Agar w/o Thienylalanine)
PKU Standard Disks
Blood test forms with Lancet
Disk test pattern for 150 mm Petri dish
150 mm Petri dishes
Forceps
Alcohol sponges
Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 50 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Simmer for 5 minutes.
3. PKU Test Agar w/o Thienylalanine, only: Add 1 ml of 0.33% ß-2-thienylalanine solution per liter after simmering the medium; mix thoroughly.
4. Dispense 150 ml amounts into flasks.
5. Aseptically add 1 ml Subtilis Spore Suspension No. 2 to each 150 ml aliquot at 50-55°C. Mix thoroughly to uniformly distribute the spores.

Specimen Collection and Preparation

1. Obtain the sample at least 48 hours after the first milk feeding.
2. Collect a venous blood sample by heel puncture following established collection technique.[9] Obtain sufficient blood to fill each circle by a single application of the specimen card to the drop of blood. Completely saturate the entire circle to ensure accuracy. Allow the blood sample to air dry.
3. Punch a 1/4" disk from one of the blood spots and place it into a labeled, clean, dry vial or place the entire specimen card on a wire rack in the autoclave.
4. Autoclave the patient disks for exactly three minutes at 121°C. Remove the disks promptly after the temperature has dropped below 100°C. Do not use the disks until they are dry.
5. Follow manufacturer's instructions for preparation of the control disks.

Test Procedure

1. Prepare PKU Test Agar or PKU Test Agar w/o Thienylalanine per label directions.
2. Dispense the final medium into 150 mm Petri dishes. Allow to solidify.
3. Using clean forceps, apply the autoclaved and dried test disks and the prepared PKU Standard Disks, one of each concentration, to the PKU Test Agar and press down gently.
4. Incubate at 35°C for 12-16 hours.
5. Compare zones of growth around the patient disks to those around the control disks to determine the approximate concentration of phenylalanine in the blood.

Results

Growth zone diameters around the control disks are related to the concentration of phenylalanine in the disks. A zone of growth may or may not be present around the test disks depending on the presence or absence of phenylalanine in the test specimen. The culture medium outside the zones of growth will be comparable to an inoculated and incubated plate to which no disks have been applied.

Compare the zone of growth around a test disk to the zone around the standard disk containing 4 mg% phenylalanine. If the test zone is equal to or larger than the 4 mg% control zone, the test result is a presumptive positive and should be confirmed using a second sample. If the second sample gives a similar result, determine the serum phenylalanine concentration by either a chemical[10] or a spectrofluorometric procedure.

Limitations of the Procedure

1. Collect the blood sample with care. The sample must saturate the paper. Do not allow contact between the absorbent specimen card and the collector's hands.
2. Autoclaved samples must be dry before use.
3. PKU Test Agar must not be overheated. Bring to a boil and mix gently during heating. DO NOT AUTOCLAVE.
4. Do not add spores if the temperature of the medium is above 55°C. Distribute the spores uniformly in the medium without creating bubbles.
5. Place the Petri dish on a horizontal surface while pouring the medium to ensure an even depth of agar and a uniform distribution of spores throughout the plate.
6. Test results at the 4 and 6 mg% levels are questionable and should be repeated with a second test sample and the results confirmed by a quantitative procedure.
7. Take care when opening ampules containing *B. subtilis* spores. Autoclave the emptied ampules at 121-124°C for 20 minutes.
8. Infants who are tested before 24 hours of age should have a repeat test performed by 2 weeks of age.
9. A negative test of an infant on antibiotics should be reconfirmed after antibiotic therapy is terminated. Antibiotics present in the blood sample are usually inactivated by the autoclaving procedure, but could be a source of error because some antibiotics will inhibit the growth of *B. subtilis.*[11]
10. False-negative tests can result from the submission of an inadequate sample, or if the patient has recently been exchange-transfused, or if the patient has an insufficient dietary protein load.[11]
11. False-positive results can occur.[12]

References

1. **Folling, A**. 1934. Phenylpyruvic acid as a metabolic anomaly in connection with imbecility. Z. Physiol. Chem. **227**:169-176.
2. **Jervis, G**. 1953. Phenylpyruvic oligophrenia deficiency of phenylalanine oxidizing system. Proc. Soc. Exp. Biol. Med. **82**:514-515.
3. **Guthrie, R., and H. Tiechelmann**. July 1960. London Conference on the Scientific Study of Mental Deficiency.
4. **Guthrie, R**. 1961. J. Am. Med. Assoc. **178**:863.

5. **Demain, A. L**. 1958. J. Bact. **75**:517.
6. **Guthrie, R., and A. Susi**. 1963. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics **32**:338-343.
7. **Ambrose, J. A., et al**. 1967. Clin. Chem. Acta. **15**:493.
8. **Ambrose, J. A**. 1969. Clin. Chem. **15**:15.
9. **National Committee for Clinical Laboratory Standards.** 1992. Blood collection on filter paper for neonatal screening programs, 2nd ed.; Approved Standard. LA4-A2, vol. 12, no. 13. Wayne, PA.
10. **LaDu, B. N., and P. J. Michael**. 1960. J. Lab. Clin. Med. **55**:491.
11. **Nichols, Michael J.** 1994. Tips on technology. MLO. **26**:11-12.
12. **Kirkman, H. N. , C. L. Carroll, E. G. Moore, et al.** 1982. Fifteen-year experience with screening for phenylketonuria with an automated fluorometric method. Am. J. Hum. Genet. **34**:743-752.

Packaging

PKU Test Agar	500 g	0980-17
PKU Test Agar w/o Thienylalanine	500 g	0474-17
Subtilis Spore Suspension No. 2	25 x 1	0981-36
	100 x 1	0981-84

PPLO Media

Bacto® PPLO Agar · Bacto PPLO Broth w/o CV · Bacto Mycoplasma Supplement · Bacto Mycoplasma Supplement S

Intended Use

Bacto PPLO Agar when supplemented with Bacto Mycoplasma Supplement or Bacto Mycoplasma Supplement S is used for isolating and cultivating *Mycoplasma*.

Bacto PPLO Broth w/o CV when supplemented with Bacto Mycoplasma Supplement or Bacto Mycoplasma Supplement S is used for isolating and cultivating *Mycoplasma.*

Also Known As

PPLO is an abbreviation for "pleuropneumonia-like organism."

Summary and Explanation

Members of the class Mollicutes, *Mycoplasma* was first recognized from a case of pleuropneumonia in a cow.[11] The organism was designated "pleuropneumonia-like organism," or PPLO.[11] Although some species are normal human respiratory tract flora, *M. pneumoniae* is a major cause of respiratory disease (primary atypical pneumonia, sometimes called "walking pneumonia").[11] *M. hominis*, *M. genitalium*, and *Ureaplasma urealyticum* are important colonizers (and possible pathogens) of the human genital tract.[11]

PPLO Agar was described by Morton, Smith and Leberman.[1] PPLO Agar was used in a study of the growth requirements of *Mycoplasma*,[2] along with the identification and cultivation of this organism.[3,4,5]

PPLO Broth w/o CV is prepared according to the formula described by Morton and Lecci.[2] Crystal Violet is omitted from this formula due to its inhibitory action on some *Mycoplasma*. PPLO Broth w/o CV has been used for the cultivation of *Mycoplasma* for research studies.[6,7]

Mycoplasma Supplement and Mycoplasma Supplement S are sterile desiccated enrichments for use in PPLO media as described by Hayflick.[8] The supplements are prepared according to the formulations of Chanock, Hayflick and Barile[9] and Hayflick.[10]

Principles of the Procedure

Infusion from Beef Heart and Bacto Peptone provide the nitrogen, vitamins, amino acids and carbon in PPLO Agar and PPLO Broth w/o CV. Sodium Chloride maintains the osmotic balance of these formulations. Bacto Agar, a solidifying agent, is used in PPLO Agar at a concentration slightly reduced from usual to ensure formation of the largest possible colonies because the organisms grow into the agar with only slight surface growth.[12]

PPLO media are supplemented with Mycoplasma Supplement or Mycoplasma Supplement S because *Mycoplasma* spp. are fastidious in their growth requirements.[13]

Mycoplasma Supplement contains fresh Yeast Extract and Horse Serum. Yeast Extract provides the preformed nucleic acid precursors that are required by *Mycoplasma* spp.[13] Horse Serum supplies cholesterol, a growth stimulant.[13]

Mycoplasma Supplement S is a selective enrichment prepared by adding Thallium Acetate and Penicillin to Mycoplasma Supplement. Thallium Acetate and Penicillin are selective against gram-positive and gram-negative bacteria.

Formula

PPLO Agar

Formula Per Liter

Bacto Beef Heart for Infusion, Infusion from	50 g
Bacto Peptone	10 g
Sodium Chloride	5 g
Bacto Agar	14 g

Final pH 7.8 ± 0.2 at 25°C

PPLO Broth w/o CV

Formula per Liter

Bacto Beef Heart for Infusion, Infusion from	50 g
Bacto Peptone	10 g
Sodium Chloride	5 g

Final pH 7.8 ± 0.2 at 25°C

Mycoplasma Supplement

Ingredients per 30 ml vial

Bacto Yeast Extract	0.01 g
Horse Serum, Desiccated	1.6 g

Mycoplasma Supplement S

Ingredients per 30 ml vial

Bacto Yeast Extract	0.01 g
Horse Serum, Desiccated	1.6 g
Penicillin	55,000 units
Thallium acetate	50 mg

User Quality Control

Identity Specifications

PPLO Agar

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling; solution is light to medium amber, slightly opalescent.
Prepared Medium:	Enriched w/30% Mycoplasma Supplement: light to medium amber, slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.8 ± 0.2

PPLO Broth w/o CV

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water; solution is light amber and clear to very slightly opalescent.
Prepared Medium:	Light amber, clear.
Reaction of 2.1% Solution at 25°C:	pH 7.8 ± 0.2

Mycoplasma Supplement

Lyophilized Appearance:	Straw-colored, dried button, may be dispersed.
Rehydrated Appearance:	Light to dark straw-colored, clear to slightly opalescent, readily soluble.

Mycoplasma Supplement S

Lyophilized Appearance:	Straw-colored, dried button, may be dispersed.
Rehydrated Appearance:	Light to dark straw-colored, clear to slightly opalescent solution, readily soluble.

Cultural Response

PPLO Agar, PPLO Broth w/o CV

Prepare media enriched with 30% Mycoplasma Supplement or Mycoplasma Supplement S per label directions. Inoculate PPLO Broth w/o CV and incubate at 35 ± 2°C under 5-10% CO_2 for up to 7 days. Subculture to PPLO Agar and incubate at 35 ± 2°C under 5-10% CO_2 for up to 7 days. Examine microscopically for growth on a daily basis.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycoplasma bovis	25523	100-1,000	good
Mycoplasma gallinarum	19708	100-1,000	good

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

Precautions

1. For Laboratory Use.
2. **Mycoplasma Supplement S**

   HARMFUL. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Bladder, Nerves, Kidneys, Cardiovascular System.

   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

PPLO Agar
PPLO Broth w/o CV

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

Mycoplasma Supplement
Mycoplasma Supplement S

Store the lyophilized and rehydrated supplements 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

PPLO Agar
PPLO Broth w/o CV
Mycoplasma Supplement
Mycoplasma Supplement S

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (50-60°C) (optional)
Sterile Petri dishes or tubes

Method of Preparation

PPLO Agar
PPLO Broth w/o CV

1. **PPLO Agar:** Suspend 35 grams in 700 ml distilled or deionized water and boil to dissolve completely.

   PPLO Broth w/o CV: Dissolve 21 grams in 700 mo distilled or deionized water.

2. Autoclave at 121°C for 15 minutes. Cool medium to 50-60°C.
4. Aseptically add 300 ml Mycoplasma Supplement or 300 ml Mycoplasma Supplement S to the sterile medium. Mix well.
5. Dispense as desired.

Mycoplasma Supplement
Mycoplasma Supplement S

1. Rehydrate with 30 ml sterile distilled or deionized water.
2. Rotate gently to dissolve.
3. Add 30 ml (the contents of one vial) to 70 ml sterile PPLO Agar or PPLO Broth w/o CV.

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 *Mycoplasma* spp. from clinical specimens, refer to appropriate procedures outlined in the references.[12,13,14]

Results

PPLO Agar

PPLO colonies are round with a dense center and a less dense periphery, giving a "fried egg" appearance on PPLO Agar. Vacuoles, large bodies characteristic of Mycoplasma spp., are seen in the periphery. Colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.

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. Thallium acetate can partially inhibit some mycoplasmas.[12]

References

1. **Morton, H. E., P. F. Smith, and P. R. Leberman.** 1951. Venereal diseases. Am. J. Syphilis Gonorrh. **35**:361.
2. **Morton, H. E., and J. G. Lecce.** 1953. Selective action of thallium acetate and crystal violet for pleuropneumonia like organisms of human origin. J. Bacteriol. **66**:646-649.
3. **Chanock, R. M., W. D. James, H. H. Fox, H. C. Turner, M. A. Mufson, and l. Hayflick.** 1962. Growth of Eaton PPLO in broth and preparation of complement fixing antigen. Soc. Exp. Biol. Med. **110**:884-889.
4. **Craven, R. B., R. P. Wenzel, A. M. Calhoun, J. O. Hendley, B. H. Hamory and J. M. Gwaltney, Jr.** 1976. Comparison of the sensitivity of two methods for isolation of *Mycoplasma pneumoniae*. J. Clin. Microbiol. **4**:225-226.
5. **Gregory, J. E., and K. R. Cundy.** 1970. *Mycoplasma* recovery from the male genitourinary tract: voided urine versus the urethral swab. Appl. Microbiol. **19**:268- 270.
6. **Adler, H. E., and A. J. Da Massa.** 1967. Use of formalinized *Mycoplasma gallisepticum* antigens and chicken erythrocytes in hemagglutination and hemagglutination-inhibition studies. Appl. Microbiol. **15**:245-248.
7. **Leland, D. S., M. A. Lapworth, R. B. Jones, and M. L. V. French.** 1982. Comparative evaluation of media for isolation of *Ureaplasma urealyticum* and genital *Mycoplasma* species. J. Clin. Microbiol. **16**:709-714.
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11. **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.
12. **Kenny, G. E.** 1985. Mycoplasmas, p. 407-411. *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.
13. **Taylor-Robinson, D.** 1995. *Mycoplasma* and *Ureaplasma*, p. 652-661. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
14. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

PPLO Agar	500 g	0412-17
PPLO Broth w/o CV	500 g	0554-17
	10 kg	0554-08
Mycoplasma Supplement	6 x 30 ml	0836-68
Mycoplasma Supplement S	6 x 30 ml	0837-68

Bacto® Pagano Levin Base

Intended Use

Bacto Pagano Levin Base is used with Bacto TTC Solution 1% and neomycin in isolating and differentiating *Candida* spp.

Also Known As

Pagano Levin Base is also referred to as Pagano Levin Candida Test Medium.

Summary and Explanation

Pagano Levin Base as described by Pagano, Levin, and Trejo[1] is selective for *Candida*. *Candida* spp. reduce TTC (2,3,5-triphenyltetrazolium chloride) in the medium to produce colonies with various degrees of color. Neomycin inhibits growth of most bacteria without appreciably influencing the *Candida.* Gentamicin (50 µg/ml) may also be added to reduce bacterial populations according to Yamane and Saitoh.[2] Samaranayake, MacFarlane and Williamson[3] found that modified Pagano Levin Agar was far superior to the commonly used Sabouraud Dextrose Agar in detecting multiple yeast species in a single sample.

Principles of the Procedure

Bacto Peptone provides the carbon and nitrogen required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors. Dextrose is an energy source. Bacto Agar is a solidifying agent. TTC Solution 1%, added to the basal medium, facilitates the differentiation of yeast colonies based on the color change that occurs when a microorganism reduces TTC. Neomycin added to the base inhibits the growth of most bacteria.

Formula

Pagano Levin Base

Formula Per Liter

Bacto Peptone	10 g
Bacto Yeast Extract	1 g
Bacto Dextrose	40 g
Bacto Agar	15 g

Final pH 6.0 ± 0.2 at 25°C

Precautions

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

Storage

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

User Quality Control

Identity Specifications

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

Solution: 6.6% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent.

Prepared Medium: Plain - light amber, slightly opalescent; with TTC and antibiotic - light amber, milky.

Reaction of 6.6% Solution at 25°C: pH 6.0 ± 0.2

Cultural Response

Prepare Pagano Levin Agar per label directions. Inoculate and incubate at 25-30°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Candida albicans	26790	100-1,000	good	cream to light pink
Candida krusei	6121	100-1,000	good	white, spreading
Candida stellatoidea	36232	100-1,000	good	light red
Escherichia coli	25922	1,000-2,000	inhibited	

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

Expiration Date

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

Procedure

Materials Provided

Pagano Levin Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (25-30°C)
Waterbath (50-55°C) (optional)
TTC Solution 1%
Neomycin

Method of Preparation

1. Suspend 66 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50-55°C.
5. Aseptically add 10 ml TTC Solution 1% (100 µg TTC per ml of medium and 500 µg of neomycin per ml of medium). Mix thoroughly.

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.[4-6]
2. Process each specimen, using procedures appropriate for that specimen or sample.[4-6]

Test Procedure

1. Inoculate the surface of the medium with the specimen and incubate at 25°C for 48-72 hours.

Results

C. albicans colonies appear cream-colored to light pink, smooth, round, raised, opaque and glistening. Typical *C. albicans* colonies can be confirmed on Chlamydospore Agar or Rice Extract Agar based on chlamydospore production.

References

1. **Pagano, J., J. D. Levin, and W. Trejo.** 1958. Diagnostic medium for differentiation of species of *Candida.* Antibiot. Annu. **1957-1958**:137-143.
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3. **Samaranayake, L. P., T. W. MacFarlane, and M. I. Williamson.** 1987. Comparison of Sabouraud Dextrose and Pagano-Levin Agar Media for detection and isolation of yeasts from oral samples. J. Clin. Microbiol. **25(1)**:162-164.
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5. **Baron, E. J., L. R. Peterson, S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Pagano Levin Base	500 g	0141-17

Bacto® Panthenol Assay Medium Bacto Panthenol Supplement

Intended Use

Bacto Panthenol Assay Medium is used with Bacto Panthenol Supplement in determining panthenol 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.

Panthenol Assay Medium and Panthenol Supplement, modifications of the formulas of DeRitter and Ruben,[1] are used in the microbiological assay of panthenol. *Gluconobacter oxydans* subsp. *suboxydans* ATCC® 621H is the test organism used in this assay.

User Quality Control

Identity Specifications

Panthenol Assay Medium

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	1.65% (single strength) or 3.3% (double strength) solution, soluble in distilled or deionized water on boiling. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Medium (Single-strength):	Very light amber, clear, may have a very slight precipitate.
Reaction of 1.65% Solution at 25°C:	pH 6.0 ± 0.2

Panthenol Supplement

Solution Appearance:	Colorless to very, very light amber, clear.
Reaction of Solution at 25°C:	pH 5.0-6.0

Cultural Response

Prepare Panthenol Assay Medium per label directions. Test the medium by creating a standard curve using pantoic acid reference standard at levels from 0.0 to 2.0 g per 10 ml. The medium supports the growth of *G. oxydans* subsp. *suboxydans* ATCC® 621H when prepared in single strength and supplemented with Panthenol Supplement and pantoic acid.

Principles of the Procedure

Panthenol Assay Medium with Panthenol Supplement added is a panthenol-free medium containing all other nutrients and vitamins essential for the cultivation of *G. oxydans* subsp. *suboxydans* ATCC® 621H. The addition of pantoic acid in increasing specified concentrations gives a growth response that can be measured turbidimetrically.

Formula

Panthenol Assay Medium

Formula Per Liter

Bacto Dextrose	15	g
Bacto Vitamin Assay Casamino Acids	2	g
Acid Digest of Casein	10	g
Sodium Citrate	2	g
L-Tryptophane	0.2	g
L-Cystine	0.15	g
Adenine Sulfate	10	mg
Guanine Hydrochloride	10	mg
Uracil	10	mg
ß-Alanine	2	mg
Liver Digest	0.35	mg
Nicotinic Acid	2	mg
p-Aminobenzoic Acid	2	mg
Thiamine Hydrochloride	2	mg
Riboflavin	2	mg
Pyridoxine Hydrochloride	2	mg
Folic Acid	20	µg
Biotin	16	µg
Magnesium Sulfate	0.8	g
Sodium Chloride	40	mg
Ferrous Sulfate	40	mg
Manganous Sulfate	0.16	g
Monopotassium Phosphate	2	g

Final pH 6.0 ± 0.2 at 25°C

Panthenol Supplement

Formula Per Liter

Bacto Glycerol	33	g
Sorbitan Monooleate Complex	2	g
Lactic Acid USP	0.68	g
Distilled or Deionized Water	71.5	ml

Precautions

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

Storage

Store Panthenol Assay Medium and Panthenol Supplement at 2-8°C. The dehydrated Panthenol Assay 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

Panthenol Assay Medium
Panthenol Supplement

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Gluconobacter oxydans* subsp. *suboxydans* ATCC® 621H
Sterile tubes
Sterile 0.85% saline
Distilled or deionized water
Panthenol
Pantoic Acid
Lactobacilli Agar AOAC
Incubator (30 + 2°C)
Shaker (160-300 rpm)
0.1 N NaOH
0.1 N HCl
Spectrophotometer

Method of Preparation

1. Suspend 33 grams in 900 ml distilled or deionized water.
2. Boil to dissolve.
3. Dispense 4.5 ml amounts into tubes, evenly dispersing any precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 9.5 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.
7. Aseptically add 0.5 ml Panthenol Supplement to each tube.

Specimen Collection and Preparation

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

Test Procedure

Stock cultures of *G. oxydans* subsp. *suboxydans* ATCC® 621H are grown on Lactobacilli Agar AOAC and kept in the refrigerator.

Inoculum for assay is prepared by subculturing a stock culture of G. oxydans ATCC® 621H into 10 ml of single-strength Panthenol Assay Medium supplemented with Panthenol Supplement and 4 µg/ml pantoic acid. Following incubation on a shaker (100 rpm) at 30 ± 2°C for 20-24 hours, centrifuge the culture under aseptic conditions. Decant the supernatant and wash the cells three times with sterile 0.85% saline. After the third wash, resuspend the cells in 10 ml sterile 0.85% saline and adjust to a turbidity of 65-70% transmittance when read on the spectrophotometer at 660 nm. Use one drop of this suspension to inoculate each assay flask.

A standard curve must be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated. The standard curve is obtained by using pantoic acid at levels of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6 and 2 µg per assay flask.

The concentration of pantoic acid required for the preparation of the standard curve may be prepared by the following procedure:

1. Dissolve 69.2 mg of pure panthenol in distilled water, adjust to pH 6.0 and dilute to 1 liter (1 ml contains the equivalent of 50 µg of pantoic acid).
2. Autoclave 8 ml of this solution with 8 ml 0.1 N NaOH at 121°C for 30 minutes.
3. Cool, add distilled water, adjust to pH 6.0 with 0.1 N HCl and dilute to 100 ml. This stock solution contains 4 µg of pantoic acid per ml.

Prepare the standard solution by diluting 10 ml of the stock solution with 90 ml distilled water. This standard solution contains 0.4 µg of pantoic acid per ml. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 ml per flask (50 ml).

Following inoculation, the cultures are incubated on a suitable shaker at approximately 100-300 rpm at 30 ± 2°C for 18-24 hours. Place cultures in the refrigerator to stop growth. Measure the growth turbidimetrically using a suitable spectrophotometer.

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 a medium 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. **DeRitter and Ruben.** 1949. Anal. Chem. **21**:823.

Packaging

Panthenol Assay Medium	100 g	0994-15
Panthenol Supplement	12 x 20 ml	0212-64

Bacto® Pantothenate Assay Medium

Intended Use

Bacto Pantothenate Assay Medium is used for determining the concentration of pantothenic acid and its salts by the microbiological assay technique.

Summary and Explanation

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

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

Pantothenate Assay Medium is a modification of the formula described in the United States Pharmacopeia[1] for the microbiological assay of pantothenic acid and its salts using *Lactobacillus plantarum* ATCC® 8014 as the test organism. Pantothenate Assay Medium does not contain Tween® 80 (Sorbitan Monooleate Complex), which is included in Pantothenate Medium AOAC USP.

Principles of the Procedure

Pantothenate Assay Medium is a dehydrated medium free from pantothenic acid or pantothenate but containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC® 8014.

The addition of calcium pantothenate in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige, homogeneous with a tendency to clump.
Solution:	3.65% (single-strength) or 7.3% (double- strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	(Single strength) light amber, clear, may have a slight precipitate.
Reaction of 3.65% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Prepare Pantothenate Assay Medium per label directions. Prepare a standard curve using a pantothenic acid reference standard at levels from 0.0 to 0.10 g per 10 ml. The medium supports the growth of *L. plantarum* ATCC® 8014 when supplemented with calcium pantothenate.

Formula

Pantothenate Assay Medium

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	10 g
Bacto Dextrose	40 g
Sodium Acetate	20 g
L-Cystine	0.4 g
DL-Tryptophane	0.2 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Thiamine Hydrochloride	200 µg
Riboflavin	400 µg
Niacin	1 mg
Pyridoxine	800 µg
p-Aminobenzoic Acid	200 µg
Biotin	0.8 g
Monopotassium Phosphate	1 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg

Final pH 6.7 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Bladder.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Pantothenate Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus plantarum* ATCC® 8014
Sterile test tubes
Sterile 0.85% saline
Distilled or deionized water
Calcium Pantothenate
Lactobacilli Agar AOAC
Incubator (35-37°C)
Centrifuge
0.2 N Acetic acid
0.2 N Sodium acetate
Spectrophotometer

Method of Preparation

1. Suspend 7.3 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

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

Prepare stock cultures of *L. plantarum* ATCC® 8014 in triplicate by stab inoculation of Lactobacilli Agar AOAC. Incubate cultures for 18-24 hours at 35-37°C. Store the tubes at 2-8°C. Prepare a fresh stock culture every week. Do not use a culture older than 1 week for this assay.

Inoculum

Subculture from a stock culture of *Lactobacillus plantarum* ATCC® 8014 to 10 ml of sterile single-strength Pantothenate Assay Medium supplemented with 0.02 mcg pantothenate. Incubate for 18-24 hours at 35-37°C. Centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 ml sterile 0.85% saline. After the third wash, resuspend the cells with sterile 0.85% saline and adjust to a turbidity of 40-45% transmittance when read on a spectrophotometer at 660 nm. Aseptically inoculate each assay tube with one drop of the cell suspension.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated. The standard curve is obtained by using calcium pantothenate solution at levels of 0.0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, and 0.1 µg per assay tube (10 ml). Turbidimetric determinations are made after 18-24 hours incubation at 35-37°C. Construct a standard curve and determine the concentration of the unknown by interpolation from the standard curve.

The concentration of pantothenic acid required for the preparation of the standard curve may be prepared by dissolving 50 mg dried calcium pantothenate in a solution containing approximately 500 ml distilled water, 10 ml 0.2N acetic acid and 100 ml 0.2N sodium acetate. Dilute to 1,150 ml with additional water to make the calcium pantothenate concentration 43.47 µg per ml; one ml equals 40 µg pantothenic acid.

This solution is diluted by adding 25 ml to a solution containing 500 ml distilled water, 10 ml 0.2N acetic acid and 100 ml 0.2N sodium acetate. Dilute to 1 liter with distilled water to make a stock solution containing 1.0 µg pantothenic acid per ml. The standard solution is made by diluting 2 ml of the stock solution to 100 ml with distilled water. This solution contains 0.02 µg pantothenic acid per ml. Use 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 ml per assay tube. Prepare the stock solution fresh daily.

Results

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

Limitations of the Procedure

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

References

1. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Inc., Rockville, MD.

Packaging

Pantothenate Assay Medium	100 g	0604-15

Bacto® Pantothenate Medium AOAC USP

Intended Use

Bacto Pantothenate Medium AOAC USP is used for determining the concentration of pantothenic acid and pantothenate by the microbiological assay technique.

Also Known As

AOAC is an abbreviation for Association of Official Analytical Chemists. USP is an abbreviation for United States Pharmacopeia.

Summary and Explanation

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

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

Pantothenate Medium AOAC USP is prepared for use in the microbiological assay of pantothenic acid and pantothenate according to the procedures of Calcium Pantothenate Assay in USP[1] and Pantothenate Acid Assay in AOAC.[2] *Lactobacillus plantarum* ATCC® 8014 is the test organism used in this assay.

Principles of the Procedure

Pantothenate Medium AOAC USP is a pantothenic acid/pantothenate-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *Lactobacillus plantarum* ATCC® 8014. The addition of calcium pantothenate in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige, homogeneous, tendency to clump.
Solution:	3.65% (single strength) or 7.3% (double strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	(Single strength) light amber, clear, may have a very slight precipitate.
Reaction of 3.65% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Prepare Pantothenate Medium AOAC USP per label directions. Test the medium by creating a standard curve using a pantothenic acid reference standard at 0.0 to 0.05 µg per 10 ml. The medium supports the growth of *Lactobacillus plantarum* ATCC® 8014 when prepared in single strength and supplemented with pantothenic acid.

Formula

Pantothenate Medium AOAC USP

Formula Per Liter

Bacto Dextrose	40	g
Sodium Acetate	20	g
Bacto Vitamin Assay Casamino Acids	10	g
Dipotassium Phosphate	1	g
Monopotassium Phosphate	1	g
L-Cystine	0.4	g
L-Tryptophane	0.1	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20	mg
Ferrous Sulfate	20	mg
Manganese Sulfate	20	mg
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Riboflavin	400	µg
Thiamine Hydrochloride	200	µg
Biotin	0.8	µg
p-Aminobenzoic Acid	200	µg
Nicotinic Acid	1	mg
Pyridoxine Hydrochloride	800	µg
Sorbitan Monooleate Complex	0.1	g

Final pH 6.7 ± 0.1 at 25°C

Precautions

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

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Store in a container with calcium chloride or other desiccant. Keep container tightly closed.

Expiration Date

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

Procedure

Materials Provided

Pantothenate Medium AOAC USP

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus plantarum* ATCC® 8014
Centrifuge
Sterile test tubes
Incubator (35-37°C)
Spectrophotometer (660 nm)
Calcium Pantothenate USP
0.2 N Acetic Acid
0.2 N Sodium Acetate
Distilled water

Method of Preparation

1. Suspend 7.3 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

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

Test Procedure

Follow the assay procedures as outlined in USP[1] or AOAC.[2]

Prepare stock cultures of *L. plantarum* ATCC® 8014 by stab inoculation of Lactobacilli Agar AOAC. Incubate stock cultures at 35-37°C (± 0.5°C) for 18-24 hours. Store the stock cultures at 2-8°C. Prepare fresh stab cultures every week. Do not use a culture more than one week old for preparing the inoculum.

Subculture from a stock culture of *Lactobacillus plantarum* ATCC® 8014 to a tube of sterile single-strength Pantothenate Medium AOAC USP (10 ml) supplemented with 0.2 mcg pantothenate. Incubate for 18-24 hours at 35-37°C. Centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 ml sterile 0.85% NaCI. After the third wash, resuspend the cells with sterile 0.85% NaCI and adjust to a turbidity of 40-45% transmittance when read on a spectrophotometer at 660 nm. Aseptically inoculate each assay tube with one drop of the cell suspension.

Prepare solutions of Calcium Pantothenate USP Reference Standard or pantothenic acid (or equivalent) according to USP[1] or AOAC.[2] Satisfactory results are obtained with the standard curve by using pantothenic acid at levels of 0.0, 0.005, 0.01, 0.015, 0.02 and 0.025 µg per assay tube (10 ml) for the AOAC procedure. Calcium pantothenate may be used at standard levels of 0.0, 0.01, 0.02, 0.03, 0.04 and 0.05 µg per assay tube for the USP procedure. Pantothenate Medium AOAC USP may be used for both turbidimetric and titrimetric analysis in the AOAC procedure, and for turbidimetric analysis only for the USP procedure. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C (± 0.5°C). Titrimetrically determinations are made following 72 hours incubation at 35-37°C (± 0.5°C).

The concentration of pantothenic acid or calcium pantothenate required for the preparation of the standard curve may be prepared as follows:

1. Dissolve 50 mg dried calcium pantothenate in 500 ml distilled water, 10 ml 0.2 N acetic acid and 100 ml 0.2 N sodium acetate.
2. Dilute with additional water to make calcium pantothenate concentration 43.47 µg per ml for the AOAC procedure or dilute to 50 µg per ml for the USP procedure. At 43.47 µg per ml, one ml should equal 40 µg pantothenic acid.

Dilute further by adding 25 ml of this solution to 500 ml distilled water, 10 ml 0.2 N acetic acid and 100 ml 0.2 N sodium acetate. Dilute this solution to 1 liter with distilled water to make a stock solution containing 1 µg pantothenic acid per ml. The standard solution is made by diluting 5 ml of the stock solution to 1000 ml distilled water to obtain a solution containing 0.005 µg pantothenic acid per ml. Use 0.0, 1, 2, 3, 4 and 5 ml per assay tube. For the USP procedure, dilute the 50 µg per ml solution with distilled water to make a standard concentration of 0.01 µg per ml. Other standard concentrations may be used provided the standard falls within the limits specified by USP[1] and AOAC.[2]

Results

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

Limitations of the Procedure

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

References

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

Packaging

Pantothenate Medium AOAC USP	100 g	0816-15

Bacto® Peptamin

Intended Use
Bacto Peptamin is used in preparing microbiological culture media.

Also Known As
Peptamin is also referred to as Peptic Digest of Animal Tissue.

User Quality Control

Identity Specifications

Dehydrated Appearance: Golden tan, free-flowing, granules.

Solution: 1%, 2% and 10% solutions, soluble in distilled or deionized water.

1%-Very light amber, clear to very slightly opalescent, may have a slight precipitate.

2%-Light amber, clear to slightly opalescent, may have a slight precipitate.

10%-Light to medium amber, clear to slightly opalescent, may have a slight precipitate.

Reaction of 1% Solution at 25°C: pH 7.0-7.6

Cultural Response

Add inoculum density of organism. All solutions are prepared with pH adjusted to 7.2-7.4.

TEST	SOLUTION	ORGANISM	ATCC®	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	negative
Indole Production	0.1%	*Escherichia coli*	25922*	positive
Acetyle-methylcarbinol Production	0.1% w/0.5% Dextrose	*Enterobacter aerogenes*	13048*	positive
Hydrogen Sulfide	1%	*Salmonella typhi*	6539	positive
Growth Response	2% w/0.5% NaCl, 0.1% Agar, & 0.1% Dextrose	*Brucella suis*	4314	good growth
Growth Response	2% w/0.5% NaCl, 0.1% Agar, & 0.1% Dextrose	*Escherichia coli*	25922*	good growth
Growth Response	2% w/0.5% NaCl, 0.1% Agar, & 0.1%	*Staphylococcus aureus*	25923*	good growth

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

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

Summary and Explanation
The development of Peptamin is the result of accumulated information that no single peptone is the most suitable nitrogen source for culturing fastidious bacteria. Extensive investigations were undertaken at Difco Laboratories using peptic digests of animal tissue prepared under varying digestion parameters.

Peptamin complies with the US Pharmacopeia XXIII (USP)[1] specification for peptic digest of animal tissue. Diluting and rinsing solutions, Fluid A and Fluid D, contain 0.1% Peptamin. Fluid A and Fluid D conform to the specifications of USP[1] for diluting and rinsing fluids in sterility tests.

Brucella media used for the cultivation of fastidious microorganisms contain Peptamin as the nitrogen source. Peptamin is used in Disinfectant Test Broth AOAC and Letheen Broth, media used for testing disinfectants. Media containing Peptamin are specified in standard methods for multiple applications.[2,3,4]

Principles of the Procedure
Peptamin provides nitrogen, amino acids, vitamins and carbon in microbiological culture media.

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

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

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

Procedure

Materials Provided
Peptamin

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

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

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. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Peptamin	500 g	0905-17

Bacto® Peptone
Bacto Peptone Bacteriological Technical

Intended Use

Bacto Peptone and Bacto Peptone Bacteriological Technical are used in preparing microbiological culture media.

Summary and Explanation

Bacto Peptone, an enzymatic digest of protein, was first introduced commercially in 1914 and became the standard Peptone for the preparation of bacteriological culture media. The importance of Peptone as a nutritive source in culture media was demonstrated by studies of Klinger.[5] The nutritive value of Peptone is largely dependent upon the amino acid content that supplies essential nitrogen.

Many studies have used Bacto Peptone in culture media preparation.[6,7,8,9] In a study by Morton, Smith and Leberman,[10] Bacto Peptone was reported to be superior to other peptones in a medium recommended for the isolation and cultivation of pleuropneumonia-like organisms. Bacto Peptone has been shown to be a satisfactory enrichment, replacing serum, for cell proliferation.[11] Peptone is routinely recommended for culture media preparation. Several media containing Peptone are specified in standard methods[1,2,3,4] for multiple applications.

Peptone Bacteriological Technical can be used as the nitrogen source in microbiological culture media when a standardized peptone is not essential. Although it has not been as carefully standardized as other peptones, certain parameters such as solubility, clarity, pH and other growth supporting properties are monitored to permit its use as a nitrogen source.

Principles of the Procedure

Bacto Peptone and Peptone Bacteriological Technical are enzymatic digests of protein. Bacto Peptone contains nitrogen in a form that is readily available for bacterial growth. Both products have a high peptone and amino acids content and only a negligible quantity of proteoses and more complex nitrogenous constituents.

Typical Analysis

Bacto Peptone

Physical Characteristics

Ash (%)	4.4	Loss on Drying (%)	3.0
Clarity, 1% Solution (NTU)	0.5	pH, 1% Solution	7.0
Filterability (g/cm²)	0.5		

Carbohydrate (%)

Total	6.9		

Nitrogen Content (%)

Total Nitrogen	15.5	AN/TN	20.0
Amino Nitrogen	3.1		

Amino Acids (%)

Alanine	8.67	Lysine	3.42
Arginine	6.76	Methionine	1.19
Aspartic Acid	5.60	Phenylalanine	1.81
Cystine	0.20	Proline	8.80
Glutamic Acid	10.21	Serine	2.87
Glycine	15.59	Threonine	1.81
Histidine	0.58	Tryptophan	0.36
Isoleucine	1.45	Tyrosine	0.64
Leucine	3.01	Valine	2.35

Inorganics (%)

Calcium	0.008	Phosphate	0.445
Chloride	1.086	Potassium	0.203
Cobalt	<0.001	Sodium	1.759
Copper	<0.001	Sulfate	0.244
Iron	0.004	Sulfur	0.410
Lead	<0.001	Tin	<0.001
Magnesium	0.007	Zinc	0.001
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	<0.5
Choline (as Choline Chloride)	2000.0	Pantothenic Acid	5.9
Cyanocobalamin	<0.1	Pyridoxine	1.7
Folic Acid	0.3	Riboflavin	3.9
Inositol	2400.0	Thiamine	<0.1
Nicotinic Acid	21.9	Thymidine	413.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	273
Salmonella	negative	Thermophile Count	13
Spore Count	90		

Precautions

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

User Quality Control

Identity Specifications

Bacto Peptone

Dehydrated Appearance: Tan, free-flowing granules.

Solution: 1%, 2% and 10% solutions are soluble in distilled or deionized water:
1%- Light amber, clear, no precipitate;
2%- Light to medium amber, clear, no precipitate;
10%- Medium to dark amber, clear to very slightly opalescent, may have a very slight precipitate.

Reaction of 1% Solution at 25°C: pH 6.8-7.2

Peptone Bacteriological Technical

Dehydrated Appearance: Tan, free-flowing, granules.

Solution: 1%, 2% and 10% solutions are soluble in distilled or deionized water:
1%- Very light to light amber, clear;
2%- Light-medium amber, clear;
10%- Medium-dark amber, clear to very slightly opalescent.

Reaction of 1% Solution at 25°C: pH 6.3-7.6

Cultural Response

Bacto Peptone

All solutions are adjusted to pH 7.2-7.4.

TEST	SOLUTION	ORGANISM	ATCC®	INOCULUM	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*		negative
Indole Production	0.1%	*Escherichia coli*	25922*		positive
Acetylmethyl-carbinol Production	0.1% with 0.5% Dextrose	*Enterobacter aerogenes*	13048*		positive
Hydrogen Sulfide Production	1%	*Salmonella typhi*	6539		positive
Growth Response	2% with 1.5% Agar and 0.5% NaCl	*Escherichia coli*	25922*	100-1,000	good growth
Growth Response	2% with 1.5% Agar and 0.5% NaCl	*Staphylococcus aureus*	25923*	100-1,000	good growth

Peptone Bacteriological Technical

Prepare 2% Peptone Bacteriological Technical in 0.5% saline and adjust to pH 7.2-7.4; add 1.5% Bacto Agar, boil and sterilize. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

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

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

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Bacto Peptone
Peptone Bacteriological Technical

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Bacto Peptone or Peptone Bacteriological Technical in the formula of the medium being prepared. Add Bacto Peptone or Peptone Bacteriological Technical 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 Bacto Peptone or Peptone Bacteriological Technical.

Results

Refer to appropriate references and procedures for results.

References

1. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 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. **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.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Klinger, I. J.** 1917. The effect of hydrogen ion concentration on the production of precipitates in a solution of peptone and its relation to the nutritive value of media. J. Bacteriol. **2**:351-353.
6. **Spray, R. S.** 1929-1930. J. Lab. Clin. Med. **15**:179.
7. **Stainsby and Nicholls.** 1932. J. Lab. Clin. Med. **17**:530.
8. **Huntoon, F. M.** 1918. "Hormone" medium. A simple medium employable as a substitute for serum medium. J. Infect. Dis. **23**:169-172.

9. **Jones and Wise.** 1926. J. Bacteriol. **11**:359.
10. **Morton, H. E., P. F. Smith, and P. R. Leberman.** 1951. Venereal diseases. Am. J. Syphilis Gonorr. **35**:361.
11. **Rutzky, L. P.** 1981. Peptone growth factors for serial cell proliferation in the absence of serum. Cambridge University Press.

Packaging

Bacto Peptone	100 g	0118-15
	500 g	0118-17
	2 kg	0118-07
	10 kg	0118-08
Peptone Bacteriological, Technical	500 g	0885-17

Bacto® Peptone Iron Agar

Intended Use

Bacto Peptone Iron Agar is used for detecting hydrogen sulfide production by microorganisms.

Summary and Explanation

Levine and co-workers[1,2] described a medium containing Proteose Peptone and ferric citrate for detection of hydrogen sulfide production by coliform bacteria. They demonstrated that such a medium served to differentiate strains that were Voges-Proskauer negative, methyl-red positive and citrate positive from other members of the *Enterobacteriaceae*.

Levine reported that ferric citrate was a much more sensitive indicator of hydrogen sulfide production than lead acetate, producing a medium that gave definite reactions within 12 hours. Peptone Iron Agar is a modification of Levine's original formula in which Bacto Peptone has been included with Proteose Peptone and the more soluble ferric ammonium citrate is used in place of ferric citrate.

Tittsler and Sandholzer[3] compared Peptone Iron Agar with lead acetate agar for the detection of hydrogen sulfide and found that Peptone Iron Agar had the advantage of giving earlier reactions and clearer results.

Principles of the Procedure

Bacto Peptone and Proteose Peptone are nitrogen sources in Peptone Iron Agar. Ferric Ammonium Citrate and Sodium Thiosulfate are used to detect H_2S production. Sodium Glycerophosphate is a buffering compound. Bacto Agar is a solidifying agent.

Formula

Peptone Iron Agar

Formula Per Liter

Bacto Peptone	15 g
Bacto Proteose Peptone	5 g
Ferric Ammonium Citrate	0.5 g
Sodium Glycerophosphate	1 g
Sodium Thiosulfate	0.08 g
Bacto Agar	15 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 3.6% Solution at 25°C:	pH 6.7 ± 0.2

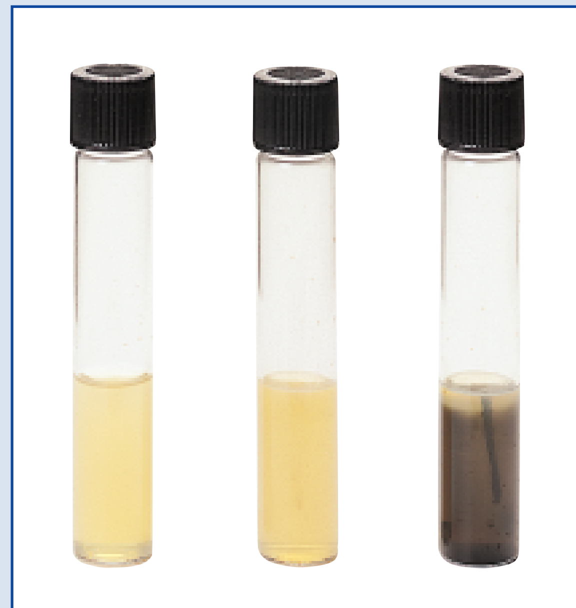
Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	H_2S PRODUCTION
Escherichia coli	25922*	undiluted	good	–
Salmonella enteritidis ser. *enteritidis*	13076	undiluted	good	+ (black)

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

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



Uninoculated tube — *Escherichia coli* ATCC® 25922 — *Salmonella enteritidis* ATCC® 13076

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

Peptone Iron Agar

Materials Required but not Provided

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

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. If preparing tubes, dispense the medium in 10 ml amounts. If preparing plates, leave the medium in the flask.
4. Autoclave at 121°C for 15 minutes.
5. If preparing tubes, cool in an upright position. If pouring plates, cool the medium to 45-50°C. 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.[4,5,6]
2. For specific information about specimen preparation and inoculation for isolation of coliform bacteria, consult appropriate references.[4,5,6]
3. Once isolated colonies of coliform bacteria are obtained on solid plated media, follow the test procedure below.

Test Procedure

1. Obtain a pure culture of a test organism. Pick the center of a single colony with an inoculating needle.
2. Inoculate a tube of Peptone Iron Agar by the stab method. Stab the needle to within 1/4 to 1/2 inch of the bottom. Withdraw the needle following the initial line of inoculation.
3. Incubate tubes at 35°C for 18-48 hours.
4. Read tubes for growth and hydrogen sulfide production.

Results

Any blackening of the medium along the line of inoculation or throughout the butt indicates hydrogen sulfide production.

For a complete discussion of the identification of coliform bacteria, refer to the appropriate references.[4,5,6]

References

1. **Levine, M., R. Vaughn, S. S. Epstein, and D. Q. Anderson.** 1932. Some differential reactions in the colon-aerogenes group of bacteria. Proc. Soc. Exp. Biol. Med. **29**:1022-1024.
2. **Levine, M., S. S. Epstein, and R. H. Vaughn.** 1934. Differential reactions in the colon group of bacteria. Am. J. Publ. Health **24:**505-510.
3. **Tittsler, R. P., and L. A. Sandholzer.** 1937. Advantages of peptone iron agar for the routine detection of hydrogen sulphide production. Am. J. Publ. Health **27:**1240-1242.
4. **Pezzlo, M. (ed.).** 1994. Aerobic bacteriology, p. 1.0.1.-1.20.47. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **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

Peptone Iron Agar	500 g	0089-17

Bacto® Peptone Water

Intended Use

Bacto Peptone Water is used for cultivating non-fastidious organisms, for studying carbohydrate fermentation patterns, and for performing the indole test.

Summary and Explanation

The formulation of Peptone Water makes it useful for cultivating non-fastidious organisms.[1] This non-selective medium has been used as a basal medium for biochemical tests such as carbohydrate fermentation patterns and production of indole.[1,2]

Principles of the Procedure

Peptone Water contains Peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium Chloride maintains the osmotic balance of the medium.

Formula

Peptone Water

Formula Per Liter

Peptone	10 g
Sodium Chloride	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Peptone Water

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Tubes with closures
Fermentation tubes
Carbohydrate solutions

User Quality Control

Identity Specifications

Dehydrated Appearance: Cream-white to light tan, free-flowing, homogeneous.

Solution: 1.5% solution, soluble in distilled or deionized water on warming with frequent agitation. Solution is light amber, clear to very slightly opalescent.

Reaction of 1.5% Solution at 25°C: pH 7.2 ± 0.2

Cultural Response

Growth/Indole Reaction

Prepare Peptone Water per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. Indole reaction is read using Indole Test Strips (1627).

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	INDOLE REACTION
Escherichia coli	25922*	undiluted	good	positive

Carbohydrate Fermentation

Prepare Peptone Water per label directions with the addition of phenol red and dextrose. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

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

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

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

1% Phenol Red solution
Indole Test strips

Method of Preparation

1. Dissolve 15 grams in 1 liter distilled or deionized water with warming and frequent agitation.
2. Autoclave at 121°C for 15 minutes.

For Determining Carbohydrate Fermentation Patterns

1. Add 1.8 ml 1% phenol red solution to 1 liter rehydrated Peptone Water. Mix thoroughly.
2. Dispense into test tubes containing inverted Durham vials.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add sufficient sterile carbohydrate solution to yield a 1% final concentration. Rotate each tube to thoroughly distribute the carbohydrate.

Specimen Collection and Preparation

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

Test Procedure

For Performing Carbohydrate Fermentation

1. Inoculate tubes with test organism.
2. Incubate tubes at 35 ± 2°C for 18-48 hours.
3. Observe for color change.

For Performing the Indole Test

1. Using aseptic technique, suspend an Indole Test Strip 10 mm above the surface of a 24 or 48 hour culture.
2. Incubate at 37°C for 5-30 minutes.

Results

For Determining Carbohydrate Fermentation Patterns

Acid is produced when carbohydrates are fermented. This is indicated by a yellow color in the medium. Gas production is indicated by the presence of gas bubbles in the fermentation tube.

For performing the Indole Test

Observe for the formation of a violet color on the strip which indicates a positive test for indole production.

Limitations of the Procedure

1. Medium is pink in color when hot but becomes colorless upon cooling.
2. *Vibrio* spp. should not be incubated longer than 18-20 hours. Longer incubation may cause the development of suppressed forms.[3]

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 610-612. Williams & Wilkins, Baltimore, MD.
2. **Balows, A., W. J. Hausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
3. **Finegold, S. M., and W. Martin.** 1982. Bailey and Scott's diagnostic microbiology, 6th ed. St. Louis

Packaging

Peptone Water	500 g	1807-17

Phenol Red Agar Media

Bacto® Phenol Red Agar Base · Bacto Phenol Red Lactose Agar Bacto Phenol Red Mannitol Agar

Intended Use

Bacto Phenol Red Agar Base is used with added carbohydrate in differentiating pure cultures of bacteria based on fermentation reactions.

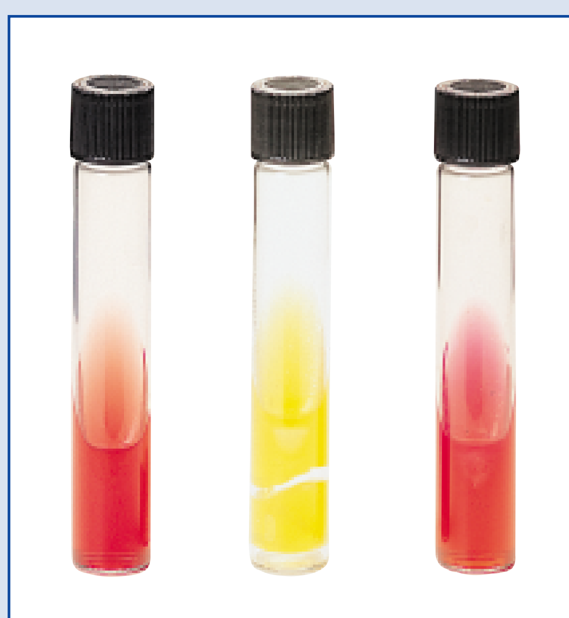
Bacto Phenol Red Lactose Agar is used for differentiating pure cultures of bacteria based on lactose fermentation reactions.

Bacto Phenol Red Mannitol Agar is used for differentiating pure cultures of bacteria based on mannitol fermentation reactions.

Summary and Explanation

Phenol Red Agar Base with added carbohydrate is well suited for the study of fermentation reactions of microorganisms.[1,2,3,4] However, while liquid media are generally employed in studying fermentation reactions, many bacteriologists prefer a solid medium for this purpose. One advantage of a solid fermentation medium is that it permits observation of fermentation reactions under both aerobic and anaerobic conditions.[5,6] Deep tubes can provide sufficiently anaerobic conditions for the growth of obligate anaerobic bacilli. Any gas formation that occurs during a reaction is indicated by splitting of the agar or accumulation of gas bubbles in the base.

Phenol Red Agar Base supports excellent growth of many fastidious bacteria. It is a basal medium free of any fermentable carbohydrates that could give erroneous interpretations. With the exception of the omitted carbohydrate, it is a complete medium prepared with Phenol Red as an indicator of reaction changes. Phenol Red Agar Base permits the user to prepare any quantity of medium needed, adding to



Uninoculated tube | Typical positive reaction with acid and gas | Typical negative reaction with positive growth

User Quality Control

Identity Specifications

Dehydrated Appearance: Pink, homogeneous, free-flowing.

Solution: **Phenol Red Agar Base:** 3.1% solution, soluble in distilled or deionized water upon boiling. Solution is orange-red to red, clear to slightly opalescent.

Phenol Red Lactose Agar: 4.1% solution, soluble in distilled or deionized water upon boiling. Solution is orange-red to red, slightly opalescent without significant precipitate.

Phenol Red Mannitol Agar: 4.1% solution, soluble in distilled or deionized water upon boiling. Solution is orange-red to red, very slightly opalescent.

Prepared Medium: Red to orange-red, slightly opalescent.

Reaction of the Solutions at 25°C: pH 7.4 ± 0.2

Cultural Response

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

ORGANISMS	ATCC®	GROWTH	PHENOL RED AGAR BASE (w/o CARBOHYDRATES) ACID	GAS	PHENOL RED AGAR BASE w/1% MALTOSE ACID	GAS	PHENOL RED AGAR BASE w/1% SUCROSE ACID	GAS	PHENOL RED AGAR BASE w/1% DEXTROSE ACID	GAS
Alcaligenes faecalis	8750	good	–	–	–	–	–	–	–	–
Escherichia coli	25922*	good	–	–	+	+	–	–	+	+
Klebsiella pneumoniae	13883*	good	–	–	+	+	+	+	+	+
Shigella flexneri	12022*	good	–	–	–	–	–	–	+	–

ORGANISMS	ATCC®	GROWTH	PHENOL RED LACTOSE AGAR ACID	GAS	PHENOL RED MANNITOL AGAR ACID	GAS
Escherichia coli	25922*	good	+	+	+	+
Salmonella typhimurium	14028*	good	–	–	+	+
Staphylococcus aureus	25923*	good	+	–	+	–

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

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

different portions any fermentable substance to be tested. Usually a 1% final concentration of a test carbohydrate is added. An entire series of carbohydrate agars can be made up readily, conveniently, and economically. Phenol Red Lactose Agar and Phenol Red Mannitol Agar already contain the specified carbohydrate.

Principles of the Procedure

Proteose Peptone No. 3 and Beef Extract provide the carbon and nitrogen required for good growth in a wide variety of organisms. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent. Phenol Red serves as a pH indicator, turning from red-orange to yellow when acid is produced during fermentation of the carbohydrate.

Formula

Phenol Red Agar Base

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Lactose Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Lactose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Mannitol Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
D-Mannitol	10 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Phenol Red Agar Base
Phenol Red Lactose Agar
Phenol Red Mannitol Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Choice of carbohydrates to be added to the basal medium
Tubes with closures

Method of Preparation

Phenol Red Agar Base

1. Suspend 31 grams in 1 liter distilled or deionized water and boil to dissolve completely.
2. When preparing 1% carbohydrate fermentation agars, dissolve 10 grams of the desired carbohydrate in the basal medium prior to sterilization
3. Autoclave at 121°C for 15 minutes.
4. Cool the medium to 45-50°C.

OR

1. Suspend 31 grams in 900 ml distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes.
3. Cool the medium to 45-50°C.
4. Aseptically add 100 ml of a sterile 10% carbohydrate solution (w/v).
5. Dispense into sterile tubes with closures.

Phenol Red Lactose Agar
Phenol Red Mannitol Agar

1. Suspend 41 grams of the selected medium in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense into tubes. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate the sterile medium by stabbing into the butt and streaking the surface of the slant.

   If desired, inoculate obligate anaerobic bacteria into melted medium that has been cooled to 45°C. Allow the agar to solidify prior to incubation.
2. Incubate at 35 ± 2°C for 4-48 hours (or anaerobically for 24-72 hours).
3. Examine periodically for growth, acid production and gas formation.

Results

Fermentation of the carbohydrate is indicated by a change in the color of the medium from red to canary yellow. Gas formation is indicated by the collection of gas bubbles in the base or by splitting of the agar.

Limitations of the Procedure

1. The addition of some carbohydrates to the basal medium may cause an acid reaction. To restore the original pH (and color of the medium), add 0.1 N sodium hydroxide on a drop-by-drop basis. Take care not to make the medium too alkaline, which would prevent fermentation from occurring within the usual incubation period.
2. When inoculating tubes, stab gently and do not use a loop. Rough stabbing or using a loop to stab may give the false appearance of gas production when mechanical splitting of the medium is what actually occurred.

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc., St. Louis, MO.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.).** 1995. Manual of clinical microbiology, 6th edition. American Society for Microbiology, Washington, D.C.
3. **Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams.** 1994. Bergey's manual of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, MD.
4. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition. Elsevier Science Publishing Co., Inc., New York, NY.
5. **Bacteriological Analytical Manual,** 8th edition. 1995. AOAC International, Gaithersburg, MD.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Phenol Red Agar Base	500 g	0098-17
Phenol Red Lactose Agar	500 g	0100-17
Phenol Red Mannitol Agar	500 g	0103-17

Phenol Red Carbohydrate Media

Bacto® Phenol Red Broth Base · Bacto Phenol Red Dextrose Broth · Bacto Phenol Red Lactose Broth · Bacto Phenol Red Mannitol Broth · Bacto Phenol Red Saccharose Broth

Intended Use

Phenol Red Carbohydrate Media are basal media used with added carbohydrates in differentiating pure cultures of bacteria based on fermentation reactions.

Summary and Explanation

The fermentative properties of bacteria are valuable criteria in their identification.[1,2,3,4] A basal medium for determining the fermentation reactions of microorganisms must be capable of supporting growth of test organisms and be free from fermentable carbohydrates. Vera[5] used a fermentation test medium employing the pH indicator phenol red and obtained highly accurate results.

Phenol Red Broth Base is recommended for use to determine the ability of organisms to ferment various carbohydrates.[6,7,8,9] Different fermentable substances may be added in any desired concentration. The concentration of carbohydrate generally employed for testing fermentation reactions of bacteria is 0.5 to 1%. Some investigators prefer to use 1% rather than 0.5% to ensure against reversion of the reaction due to depletion of the carbohydrate.

Phenol Red Broth Base is an excellent substrate for streptococci, as well as for other less fastidious bacteria, the growth promotion of the medium can be greatly improved for fastidious, microaerophilic, and obligately anaerobic strains by the addition of a small amount of Bacto Agar (0.1-0.2%). A medium containing this small quantity of agar may be heated it to the boiling point to drive out the dissolved air. The tubes are then cooled to below 40°C, without excessive agitation, just prior to inoculation. The fermentation reaction of gonococci may be determined by using 0.8% Bacto Agar and adding 5% sterile fresh rabbit serum to the sterile Phenol Red Broth Base containing the selected carbohydrate. Coagulase Plasma EDTA can be added to Phenol Red Mannitol Broth to prepare Coagulase Mannitol Broth. This medium is useful in determining the ability of *Staphylococcus aureus* to ferment mannitol and to coagulate plasma.[10]

Principles of the Procedure

Proteose Peptone No. 3 and Beef Extract provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Sodium Chloride maintains the osmotic balance of the medium. Phenol Red serves as an indicator, turning from red-orange to yellow when acid is produced during fermentation of the added carbohydrates.

Formula

Phenol Red Broth Base

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Dextrose Broth

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g
Bacto Dextrose	5 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Lactose Broth

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g
Bacto Lactose	5 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Mannitol Broth

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g
Bacto Mannitol	5 g

Final pH 7.4 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Phenol Red Broth Base

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 1.6% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.

Prepared Media: Orange-red to red, clear.

Reactions of 1.6% Solution at 25°C: pH 7.4 ± 0.2

Phenol Red Dextrose Broth

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 2.1% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.

Prepared Media: Orange-red to red, clear.

Reactions of 2.1% Solution at 25°C: pH 7.4 ± 0.2

Phenol Red Lactose Broth

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 2.1% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.

Prepared Media: Orange-red to red, clear.

Reactions of 2.1% Solution at 25°C: pH 7.4 ± 0.2

continued on following page

Phenol Red Saccharose Broth

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g
Bacto Saccharose	5 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Carbohydrate Broths contain the above ingredients with 5 g/liter of the specified carbohydrate.

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. Store the prepared media at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Phenol Red Broth Base
Phenol Red Dextrose Broth
Phenol Red Lactose Broth
Phenol Red Mannitol Broth
Phenol Red Saccharose Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Carbohydrates (as needed)
Tubes with closures
Fermentation tubes

Method of Preparation

Phenol Red Broth Base

1. Dissolve 16 grams in 1 liter distilled or deionized water.
2. Distribute into tubes. To detect gas production, place inverted fermentation tubes (Durham tubes) in the tubes of medium.
3. Autoclave at 121°C for 15 minutes.

When preparing 0.5-1% carbohydrate fermentation broths, dissolve 5-10 grams of the desired carbohydrate in the basal medium prior to sterilization, or dissolve 16 grams of Phenol Red Broth Base in 900 ml distilled or deionized water and aseptically add 100 ml of a sterile 5-10% carbohydrate solution (w/v) after sterilizing and cooling the basal medium.

Phenol Red Dextrose Broth, Phenol Red Lactose Broth, Phenol Red Mannitol Broth, Phenol Red Saccharose Broth

1. Suspend 21 grams of the appropriate Phenol Red Carbohydrate Broth in 1 liter distilled or deionized water and stir to dissolve completely.
2. For better growth of fastidious organisms (such as streptococci, pneumococci, and gonococci) add 1 gram of Bacto Agar per liter of medium and dissolve by boiling prior to sterilizing.
3. Dispense into tubes. To detect gas production, place inverted fermentation tubes in the tubes of medium.
4. Autoclave at 121°C for 15 minutes.

If the media are not used the same day they are sterilized, prior to use, place the medium in flowing steam or a boiling water bath for a few minutes to drive off dissolved gases. Allow to cool without agitation.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate tubes with one drop of a diluted pure culture.
2. Incubate at 35 ± 2°C for 4-18 hours with caps loosened.
3. Examine tubes for growth, acid production, and gas production (if fermentation vials are used).

Results

A yellow color of the medium indicates a positive reaction for carbohydrate fermentation. If fermentation vials are used, bubbles in the inverted vials are an indication of gas production. The presence of a single bubble is recorded as positive for the production of gas.[10]

Limitations of the Procedure

1. The addition of some carbohydrates to the basal medium may result in an acid reaction. In this case, it is suggested that 0.1N sodium hydroxide be added drop by drop to restore the original color. Take care not to make the medium too alkaline for true fermentation to occur within the usual incubation period.
2. To ensure accuracy of interpretation, uninoculated control tubes and/or inoculated Phenol Red Broth Base control tubes should be run in parallel with the fermentation tests.

User Quality Control cont.

Phenol Red Mannitol Broth

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 2.1% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.

Prepared Media: Orange-red to red, clear.

Reactions of 2.1% Solution at 25°C: pH 7.4 ± 0.2

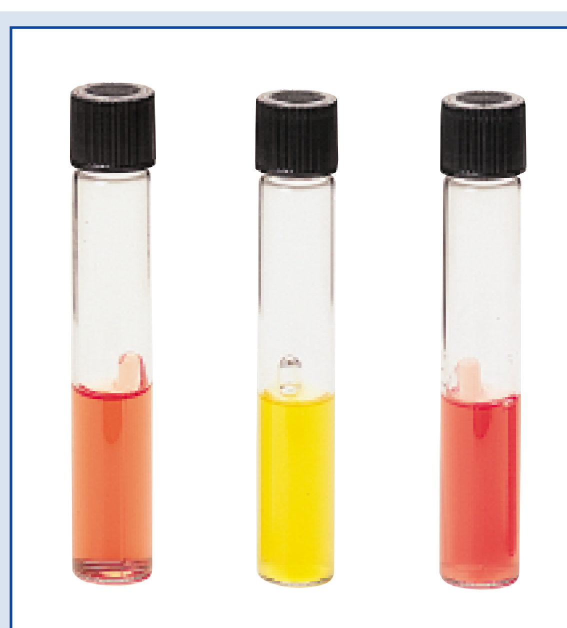
Phenol Red Saccharose Broth

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 2.1% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.

Prepared Media: Orange-red to red, clear.

Reactions of 2.1% Solution at 25°C: pH 7.4 ± 0.2



Uninoculated tube — Typical positive reaction with acid and gas — Typical negative reaction with positive growth

Cultural Response

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

ORGANISM	ATCC®	GROWTH	BASE A	BASE G	DEXTROSE A	DEXTROSE G	LACTOSE A	LACTOSE G	MANNITOL A	MANNITOL G	SACCHAROSE A	SACCHAROSE G
Alcaligenes faecalis	8750	good	–	–	–	–	–	–	–	–	–	–
Escherichia coli	25922*	good	–	–	+	+	+	+	+	+	–	–
Klebsiella pneumoniae	13883*	good	–	–	+	+	+	+	+	+	+	+
Shigella flexneri	12022*	good	–	–	+	–	–	–	+	–	–	–

A = Acid
G = Gas

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

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc., St. Louis, MO.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.).** 1995. Manual of clinical microbiology, 6th edition. American Society for Microbiology, Washington, D.C.
3. **Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams.** 1994. Bergey's manual of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, MD.
4. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition. Elsevier Science Publishing Co., Inc., New York, NY.
5. **Vera, H. D.** 1950. Relation of peptones and other culture media ingredients to accuracy of fermentation tests. Am. J. Public Health **40**:1267.
6. **Bacteriological Analytical Manual,** 8th edition. 1995. AOAC International, Gaithersburg, MD.
7. **Vanderzant, C., and D. F. Splittstoesser.** 1992. Compendium of methods for the microbiological examination of foods. American Public Health Assoc., Washington, D.C.
8. **Association of Official Analytical Chemists.** 1995 official methods of analysis of AOAC International. AOAC International, Arlington, VA.
9. **Franson, M. A. H., A. D. Eaton, L. S. Clesceri, and A. E. Greenberg.** 1995. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C.
10. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Phenol Red Broth Base	500 g	0092-17
Phenol Red Dextrose Broth	500 g	0093-17
Phenol Red Lactose Broth	500 g	0094-17
Phenol Red Mannitol Broth	500 g	0097-17
Phenol Red Saccharose Broth	500 g	0095-17

Bacto® Phenylalanine Agar

Intended Use

Bacto Phenylalanine Agar is used for differentiating *Proteus* and *Providencia* species from other *Enterobacteriaceae* based on deamination of phenylalanine.

Also Known As

Phenylalanine Agar is also known as Phenylalanine Deaminase Medium.

Summary and Explanation

Buttiaux, Osteux, Fresnoy and Moriamez[1] developed a method to differentiate members of the *Proteus* and *Providencia* groups from

User Quality Control

Identity Specifications

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

Solution: 2.3% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent without significant precipitate.

Prepared Medium: Light amber, slightly opalescent without precipitate.

Reaction of 2.3% Solution at 25°C: 7.3 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	REACTION
Enterobacter aerogenes	13048*	100-1,000	good	–
Proteus vulgaris	13315*	100-1,000	good	+
Providencia alcalifaciens	9886	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 with reagent

Proteus vulgaris ATCC® 13315

other *Enterobacteriaceae* based on the ability of *Proteus* and *Providencia* to deaminate phenylalanine to phenylpyruvic acid by enzymatic activity.[2] Bynae modified this method by incorporating phenylalanine in the medium used to grow the organisms. Ewing, Davis and Reavis[4] simplified the Bynae formulation by omitting proteose peptone. Phenylalanine Agar is prepared according their formula.

Phenylalanine Agar is used to differentiate *Proteus, Providencia* and *Morganella* (originally classified in the genus *Proteus*) from other members of the family *Enterobacteriaceae.* In addition, some strains of *Enterobacter agglomerans, Enterobacter sakazakii, Rahnella aquatilis, Tatumella ptyseos* and a few nonfermenting gram-negative bacilli are also capable of deaminating phenylalanine.[4,5]

Principles of the Procedure

Phenylalanine Agar contains DL-Phenylalanine which serves as a substrate for deamination to phenylpyruvic acid. After incubation, phenylpyruvic acid is detected by the addition of ferric chloride reagent. The ferric ions chelate the phenylpyruvic acid and form a green color.[5] Yeast Extract provides vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon. Dipotassium Phosphate provides buffering capability. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Formula

Phenylalanine Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Dipotassium Phosphate	1 g
Sodium Chloride	5 g
DL-Phenylalanine	2 g
Bacto Agar	12 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

Phenylalanine Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
SpotTest™ Ferric Chloride Reagent (3557) or 8-12% ferric chloride
0.1 N HCl

Method of Preparation

1. Suspend 23 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes. Autoclave at 121°C for 15 minutes.
4. Allow medium to solidify in a slanted position.

Test Procedure

1. Inoculate the medium and incubate at 35°C for 18-24 hours.
2. After recording the growth response, add 3-5 drops of SpotTest™ Ferric Chloride Reagent to each tube.
3. Examine for color development within 1-5 minutes. A dark green color indicates a positive reaction.

Results

Positive: Dark green

Negative: No color change

Limitations of the Procedure

1. A positive phenylalanine reaction should be interpreted quickly because the green color disappears within 10 minutes after addition of ferric chloride solution. Adding additional reagent usually regenerates the color.
2. Certain species rapidly deaminate phenylalanine, allowing for a positive test result within 4 hours of incubation.[4]

References

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

Phenylalanine Agar	100 g	0745-15
	500 g	0745-17

Bacto® Phenylethanol Agar

Intended Use

Bacto Phenylethanol Agar is used for isolating staphylococci and streptococci from specimens containing gram-negative organisms.

Also Known As

Phenylethanol Agar is also referred to as Phenylethyl Alcohol (PEA) Agar.

Summary and Explanation

Brewer and Lilley[1,2] reported that the addition of phenylethanol to a nutritive medium will permit growth of gram-positive organisms but markedly to completely inhibit growth of gram-negative organisms found in the same specimen. Phenylethanol Agar inhibits the swarming of *Proteus* spp. and can be used to selectively isolate anaerobic bacteria from clinical specimens with mixed flora. Phenylethanol Agar is specified for use in several reference methods.[3,4,5]

Principles of the Procedure

Tryptose and Beef Extract provide the nitrogen and carbon required for good growth of a wide variety of organisms. Sodium Chloride maintains the osmotic balance. Bacto Agar is a solidifying agent. Phenylethanol is bacteriostatic for gram-negative bacteria and inhibits DNA synthesis. Optional addition of 5% defibrinated sheep blood to the basal medium can enhance microorganism recovery on the medium.

Formula

Phenylethanol Agar

Formula Per Liter

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

Final pH 7.3 ± 0.2 at 25°C

Precautions

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

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, homogeneous with soft clumps.
Solution:	3.55% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Medium:	Without blood - light amber, slightly opalescent; With blood - cherry red, opaque.
Reaction of 3.55% Solution at 25°C:	pH 7.3 ± 0.2

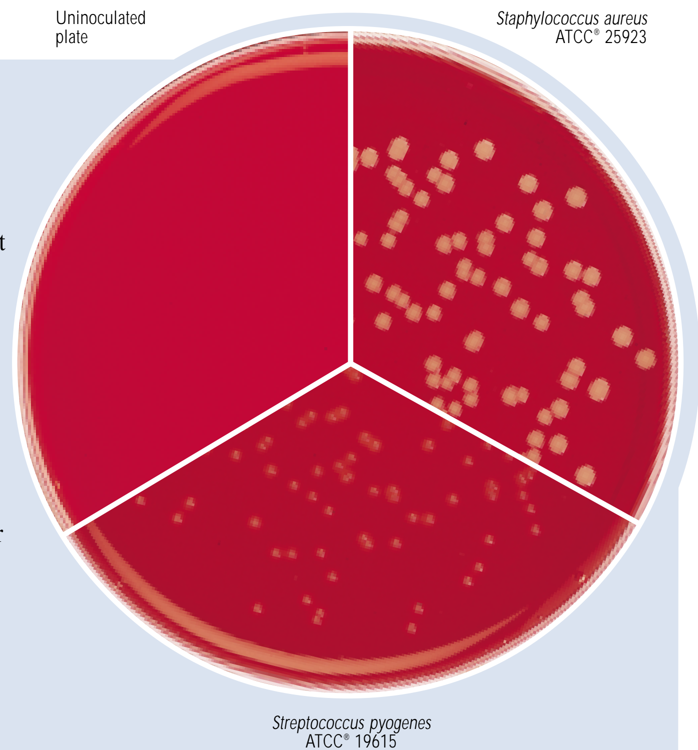
Cultural Response

Prepare Phenylethanol Agar with 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	RECOVERY	HEMOLYSIS
Proteus mirabilis	12453	1,000-2,000	partial inhibition	N/A
Staphylococcus aureus	25923*	100-1,000	growth	beta
Streptococcus pneumoniae	6305	100-1,000	growth	alpha
Streptococcus pyogenes	19615*	100-1,000	growth	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.



Storage

Store the dehydrated medium at 2-8°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

Phenylethanol Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 35.5 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the cooled medium at 45-50°C. Mix well.

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.[3-5]
2. Process each specimen using procedures appropriate for that specimen or sample.[3-5]

Test Procedure

1. Inoculate plates with test specimens. Streak to obtain isolated colonies.
2. Incubate plates at 35 ± 2°C under 5-10% CO_2 for 18-24 hours and, if necessary, 40-48 hours.

Results

Examine plates for growth and hemolysis. Perform additional biochemical testing to identify the organism.

Limitations of the Procedure

1. Some gram-positive cocci may be slightly inhibited and may require further incubation (to 48 hours) for sufficient growth to be evident.[6]
2. Subculture gram-positive colonies onto Tryptic Soy Agar (TSA), Selenite Broth and other biochemical media for definitive identification.[6]
3. *Pseudomonas aeruginosa* is not inhibited on this medium.[7]

References

1. **Brewer, J. H., and B. D. Lilley.** 1949. Paper presented at the December meeting of the Maryland Association of Medical and Public Health Laboratories.
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3. **Baron, E. J., and S. M. Finegold.** 1990. Bailey & Scott's diagnostic microbiology, 8th ed. The C.V. Mosby Company, St. Louis, MO.
4. **Isenberg, H. D.** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
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Packaging

Phenylethanol Agar	100 g	0504-15
	500 g	0504-17
	2 kg	0504-07

Phytohemagglutinins

Bacto® Phytohemagglutinin M · Bacto Phytohemagglutinin P

Intended Use

Bacto Phytohemagglutinin M and Bacto Phytohemagglutinin P are used for the isolation of lymphocytes and nucleated erythrocytes from blood and marrow. They are also used for initiating mitosis in lymphocytes for chromosomal analysis.

Also Known As

Phytohemagglutinin M is also known as PHA-M. Phytohemagglutinin P is also known as PHA-P.

Summary and Explanation

Hemagglutination

Phytohemagglutinins M or P were originally used for hemagglutination techniques.[1,2] Phytohemagglutinins were then used with dextran[3] and fibrinogen[4] to produce excellent yields of morphologically and physiologically intact lymphocytes in a suspension with no hemolysis.

Phytohemagglutinin M or P have been used to agglutinate the erythrocytes of all human blood groups, and those of many animals such as rabbit,

dog, cat, chicken, duck, mouse, rat, sheep, horse, pig, frog and guinea pig. Phytohemagglutinin has been used to obtain the plasma suspension of trypanosomes from the blood of infected rats.[5]

Mitogenic Activity

Nowell[6] discovered that phytohemagglutinin M initiates mitosis in cultures of lymphocytes isolated from peripheral blood. Later, phytohemagglutinin P was also shown to possess this property. The application of this technique is important in the characterization of chromosomes. A procedure using phytohemagglutinin-stimulated lymphoblasts has been used to cultivate human immunodeficiency virus type 1 (HIV-1) from infected individuals by cocultivation cultures.[7] Human T-lymphocytes have been activated by phytohemagglutinin to the blastic killer-cell state in preparation for in-vivo immunotherapy trials in donor cancer patients.[8]

A simplified procedure for lymphocyte mitogenesis was developed by Moorhead, Nowell, Mellman, Batipps and Hungerford,[9] in which the cultures were routinely allowed to incubate for 3 days (65-70 hours). Their method incorporated the hypotonic treatment developed by Hughes[10] and Hsu and Pomerat.[11] The flame drying of slides by Scherz[12] and the staining procedure by Rothfels and Siminovitch[13] were helpful contributions in this procedure. Staining of chromosomes by one of many methods produces characteristic bands. For more information on chromosome staining, please refer to appropriate references.[14-17]

Principles of the Procedure

Both Phytohemagglutinin M and P will agglutinate the erythrocytes of all human blood types, and those of animals. The rehydrated P-form has approximately 40 times more hemagglutinating potency than the M-form. Both forms will also stimulate the lymphocytes of peripheral blood to undergo mitosis in vitro.

Reagents

Phytohemagglutinin M is a stable, nontoxic, desiccated mucophytohemagglutinin.

Phytohemagglutinin P is a sterile, desiccated, purified, highly potent protein phytohemagglutinin from which the polysaccharide moiety has been removed.

User Quality Control

Identity Specifications

Phytohemagglutinin M or P

Lyophilized Appearance: White, porous lyophilized cake.

Solution Appearance: Contents of 1 vial, soluble in 5 ml sterile distilled or deionized water within 2 minutes. Solution is colorless, clear to slightly opalescent.

Performance Response

When reconstituted with 5 ml sterile distilled or deionized water, 0.1 ml Phytohemagglutinin M or 0.01 ml Phytohemagglutinin P is added to 7 ml RPMI #1640 Medium containing the lymphocytes from 5 ml heparinized human blood. The mitogenicity test is performed using the above components and procedures with 4 samples of human blood. A mitotic index of at least 75 should be obtained from the lymphocytes of each of the four samples of blood. A total of at least 400 should be obtained from the sum of all four cultures.

Precautions

1. For Laboratory Use.
2. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[18,19]
3. Practice the following routine laboratory safety procedures:
   Do not pipette by mouth.
   Use aseptic technique and established laboratory procedures in handling and disposing of infectious materials.

Storage

Store desiccated Phytohemagglutinin M and Phytohemagglutinin P at 2-8°C. The rehydrated solutions are stable for at least 2 weeks at -20°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

Phytohemagglutinin M
Phytohemagglutinin P

Materials Required But Not Provided

Sterile syringe
Sterile test tube
30 units sterile heparin dissolved in 0.85% sterile saline
RPMI Medium #1640
700 units of Penicillin
700 µg Streptomycin
Colchicine (10^{-5} Molar)
Hanks Balanced Salt Solution
Methanol, reagent grade
Glacial Acetic Acid, reagent grade
Deionized water
Giemsa Stain
Pipettes, 0.1 ml, 1 ml, 5 ml
Water aspirator
Pasteur pipettes
Centrifuge
Incubator, 35°C
Microscope slides
Microscope (12.5X eyepiece with 10X low power, 40X high dry, and 100X oil immersion objectives)

Reagent Preparation

Phytohemagglutinin M or the sterile Phytohemagglutinin P is rehydrated by adding 5 ml of sterile distilled or deionized water, or equivalent, and rotating gently to mix contents thoroughly. The solutions are approximately 1% in 0.85% saline. Both solutions contain approximately 50 mg protein per 5 ml.

Specimen Collection and Preparation

For each culture, a 5 ml sample of blood is adequate. Draw the blood with a sterile syringe and immediately place in a sterile screw-capped test tube containing 30 units of sterile heparin and mix thoroughly. Dissolve the heparin in 1 ml of a sterile 0.85% saline solution before collecting the specimen. Start the agglutination and mitotic procedures immediately, or they may be postponed for at least 24 hours, if the specimen is stored at 2-8°C.

Observe aseptic technique from the collection of the blood sample until the addition of the colchicine.

Test Procedure

Lymphocyte Separation and Inoculation

1. Transfer 5 ml of blood containing 30 units of heparin to a sterile screw-capped test tube under aseptic conditions.
2. Add either 0.1 ml of rehydrated Phytohemagglutinin M or 0.0025 ml of Phytohemagglutinin P to the 5 ml of heparinized blood, and mix the contents by inverting several times.
3. Let the erythrocytes agglutinate at 25°C for 15-30 minutes.
4. Centrifuge the tube at 500 rpm for 2 minutes. Excessive centrifuging must be avoided to prevent sedimentation of the lymphocytes.
5. Transfer the hazy plasma-lymphocyte suspension (about 2 ml) by means of a sterile Pasteur pipette to 7 ml of a culture medium consisting of RPMI #1640 Medium, 700 units of Penicillin, 700 µg Streptomycin, and either 0.1 ml of Phytohemagglutinin M, if the erythrocytes have been agglutinated with the M-form, or 0.01 ml of Phytohemagglutinin P, if the erythrocytes have been agglutinated with the P-form. The optimal concentration of lymphocytes in the culture is 1.0-1.2 X 10^6 per ml. If Phytohemagglutinin P and aseptic conditions are used, the antibiotics may be omitted.

Incubation of Culture

6. Incubate the culture in a vertical position at 35 ± 2°C with occasional swirling for 3-4 days. Care should be taken to maintain proper incubation temperature. A significant increase in mitotic index is often obtained by incubating 4 days instead of 3. It is very important to always maintain the proper pH range in the culture. The phenol red indicator should not become more acidic than a light amber nor more alkaline than a light pink. If the indicator becomes amber, loosen the cap for an hour or so to allow the escape of CO_2. This precaution is often most necessary at the beginning and end of the incubation.
7. End the mitosis by the addition of 1 ml of 10^{-5} molar colchicine, and continue the incubation at 35 ± 2°C for another 4-6 hours. The exposure of cells to the colchicine should not be less than 4 hours or more than 6 hours.

Harvesting and Fixation of Cells

8. Transfer the entire culture to a graduated conical centrifuge tube (15 ml) and centrifuge for 6-8 minutes at 600-800 rpm.
9. Carefully aspirate off the supernatant fluid.
10. Add 5 ml of warm (35 ± 2°C) Hanks Balanced Salt Solution and resuspend the cells in the centrifuge tube with a Pasteur pipette.
11. Centrifuge at 600-800 rpm for 6-8 minutes.
12. Carefully aspirate off the supernatant with the pipette and add 1 ml of Hanks Balanced Salt Solution.
13. Resuspend the packed cells with the Pasteur pipette.
14. Add 3 ml of warm (35 ± 2°C) distilled water, in 1 ml portions, with momentary agitation after each addition to produce a hypotonic solution.
15. Incubate the suspension at 35 ± 2°C for 10 minutes only. The exposure of the cells to this hypotonic, diluted Hanks Balanced Salt Solution should not exceed 10 minutes.
16. Centrifuge the lymphocyte solution at 600-800 rpm for 6-8 minutes.
17. Carefully aspirate off the supernatant.
18. Add slowly, without disturbing the button of cells, 4 ml of freshly prepared fixative consisting of 1 part glacial acetic acid and 3 parts methanol (reagent grade only).
19. Let the cells soak in the fixative for 15-30 minutes. Cells should be treated gently during this stage of fixation. At this point, cells may be stored overnight at 2-8°C.
20. Resuspend with the Pasteur pipette.
21. Centrifuge at 600-800 rpm for 6-8 minutes, and carefully remove the supernatant by aspiration.
22. Resuspend the cells in 4 ml fresh fixative with the Pasteur pipette, and centrifuge at 600-800 rpm for 6-8 minutes. Repeat this step again if necessary to disperse clumps of cells.
23. Carefully aspirate the supernatant.
24. Add 0.5-1.0 ml of fresh fixative to the button of cells and resuspend with the Pasteur pipette to get a hazy suspension.

Preparation of Slides

25. Label clean microscope slides and place them in clean, chilled distilled water.
26. In rapid succession, shake the excess water off a chilled slide, wipe the water off its underside, add 3-4 drops of the cell suspension by means of the Pasteur pipette, tip the slide several times to spread the suspension, and ignite the fixative by bringing it momentarily in contact with a flame. When the fixative is burned off, wave the slide vigorously to hasten drying. The slide should not get hot, but drying should be accomplished as rapidly as possible.

Staining of Slides

Slides may be stained with Giemsa, orcein or other stains according to the method of Rothfels and Siminovitch.[14] The procedure using Giemsa is given below.

27. Dilute the 1 ml of stock Giemsa Stain (20X stock) with 19 ml of distilled water. The 1 ml of stock Giemsa Stain should be used the same day it is diluted 20-fold with water.
28. Place the slides in a small staining dish or Petri dish and cover them with 20 ml of the staining solution for 10-20 minutes.
29. Rinse the slides gently in distilled water and air dry.
30. Examine the slides under the microscope. The mitotic spreads may be scanned at a total magnification of 125X, examined more closely at 500X, or photographed under oil immersion at 1,000X. Slides may be protected by cover slips and made permanent by conventional procedures.

Alternatively, the chromosomes may be treated by staining procedures to show G-banding. Refer to appropriate references for alternative staining procedures.[18]

Results

A mitotic index of at least 30 may be expected from the lymphocytes from the heparinized peripheral blood of a healthy individual.

Limitations of the Procedure

1. For mitotic investigations, avoid the following:
   › Anticoagulants containing oxalates or phenols
   › Cytotoxic antibiotics, drugs or heavy metals (Penicillin and Streptomycin are acceptable.)
   › Hypertonic and hypotonic media except for the intentional swelling of the chromosomes
   › Irradiation of the patient or culture, which can produce "breaks" in the chromosomes.
   › Some plastic materials cause cytotoxic effects.

References

1. **Li, J. G., and E. E. Osgood.** 1949. A method for the rapid separation of leukocytes and nucleated erythrocytes from blood or marrow with a phytohemagglutinin from red beans (*Phaseolus vulgaris*). Blood **4:**670-675.
2. **Takikawa, K., T. Ito, J. Kato, T. Yoshida, H. Kondo, and I. Miyata.** 1957. Studies on the isolation of granules and mitrochondria of leukocytes. Acta Haemat. **18:**179-184.
3. **Chen, H. P., and G. K. Palmer.** 1958. A method for isolating leukocytes. Am. J. Clin. Pathol. **30:**567-569.
4. **Skoog, W. A., and W. S. Beck.** 1956. Studies on the fibrinogen, dextran, and phytohemagglutinin methods of isolating leukocytes. Blood **11:**436-54.
5. **Yaeger, R. G.** 1960. A method of isolating trypanosomas from blood. J. Parasitol. **46:**288.
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13. **Rothfels, K. H., and L. Siminovitch.** 1958. An air-drying technique for flattening chromosomes in mammalian cells grown in vitro. Stain Tech. **33:**73-77.
14. **Bird, B. R., and F. T. Forrester.** 1981. Basic Laboratory Techniques in Cell Culture. U. S. Department of Health and Human Services, CDC, Atlanta, GA.
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18. **Centers for Disease Control.** 1988. Update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood borne pathogens in health-care settings. Morbidity and Mortality Weekly Reports **37:**377-382, 387-388.
19. **Occupational Safety and Health Administration, U.S. Department of Labor.** 1991. 29 CFR part 1910. Occupational exposure to blood borne pathogens; final rule. Federal Register **56:**64175-64182.

Packaging

Phytohemagglutinin M	5 ml	0528-56*
	6 x 5 ml	0528-57*
Phytohemagglutinin P	5 ml	3110-56*
	6 x 5 ml	3110-57*

*Store at 2-8°C

Bacto® Plate Count Agar
Bacto Standard Methods Agar

Intended Use

Bacto Plate Count Agar is a Standard Methods medium used for enumerating aerobic bacteria in water, wastewater, foods and dairy products.[1,2,3,4,5] This medium is also recommended as a general plating medium for determining bacterial populations.

Also Known as

Standard Methods Agar and Tryptone Glucose Yeast Agar are alternate names for Plate Count Agar.

Summary And Explanation

Plate Count Agar was developed by Buchbinder, Baris and Goldstein[6] in 1953 at the request of the American Public Health Association. Results showed that a dehydrated milk-free medium containing 0.25% Yeast Extract, 0.5% Tryptone, 0.1% Dextrose and 1.5% Agar per liter approximated the productivity of Tryptone Glucose Extract Agar with added milk. Buchbinder *et al.* recommended that a dehydrated culture medium be used in preparing the standard plate count medium rather than preparing the medium from ingredients. Bacto Plate Count Agar is prepared with the same ingredients originally suggested by Buchbinder *et al.*[7] Combinations of Yeast Extract and Tryptone have been used in media for the examination of dairy products for the presence of thermophilic organisms since 1928.[8,9] This formula is specified in *Standard Methods for the Examination of Water and Wastewater*,[1]

Standard Methods for the Examination of Dairy Products,[2] *Compendium of Methods for the Microbiological Examination of Foods*[3] and the *Association of Official Analytical Chemists (AOAC)*[4] and the *FDA Bacteriological Analytical Manual*.[5]

Principles of the Procedure

Plate Count Agar contains Tryptone and Yeast Extract which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Dextrose is a source of fermentable carbohydrate (energy source). Bacto Agar is a solidifying agent.

Formula

Plate Count Agar

Standard Methods Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	2.5 g
Bacto Dextrose (Glucose)	1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

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

Storage

Store Plate Count Agar below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store Standard Methods Agar at 15-30°C.

Expiration Date

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

Procedure

Materials Provided

Plate Count Agar
Standard Methods Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Waterbath (optional)

Method of Preparation

Plate Count Agar

1. Suspend 23.5 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Standard Methods Agar (prepared)

1. Loosen the caps on the bottles prior to heating.
2. Heat the medium in the autoclave for 7 minutes to melt the agar. A small solidified mass remains that can be melted by swirling the hot agar. Cycle time depends on the number of bottles in the chamber.

User Quality Control

Identity Specifications

Plate Count Agar

Dehydrated Medium:	Light beige, homogeneous, free-flowing.
Solution:	2.35% solution, soluble in distilled or deionized water on boiling; light amber, slightly opalescent, no precipitate.
Prepared Medium:	Light amber, slightly opalescent, no precipitate.
Reaction of 2.35% Solution at 25°C:	7.0 ± 0.2

Cultural Response

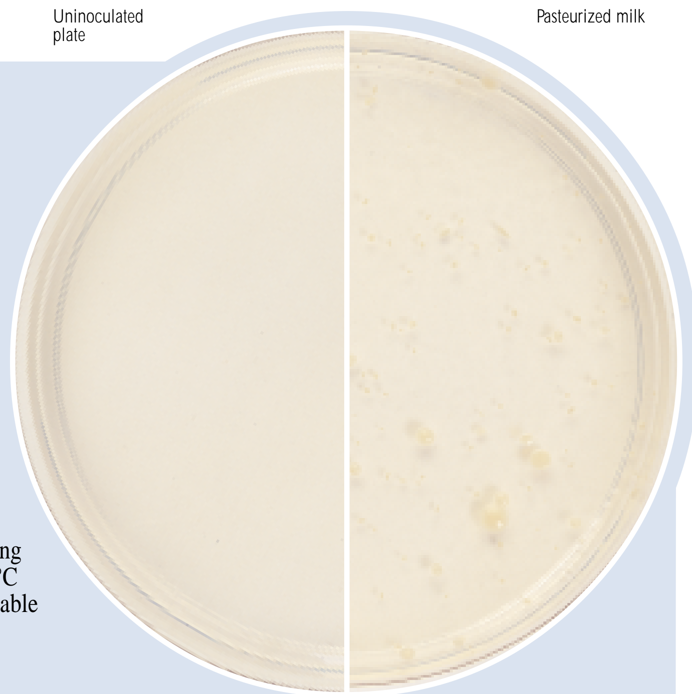
Plate Count Agar (dehydrated)

Prepare Plate Count Agar per label directions. Inoculate with serial dilutions (30-300 CFU/ml) of pasteurized and raw milk samples using the pour plate method (standard plate count) and incubate at 32 ± 1°C for 48 hours. Statistical analysis of data should yield counts comparable to an approved lot of medium.

Standard Methods Agar (prepared)

Melt Standard Methods Agar and aseptically dispense into Petri dishes. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus acidophilus	11506	30-300	good
Staphylococcus aureus	25923	30-300	good



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

Specimen Collection and Preparation

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

Test Procedure

1. Perform serial dilutions on samples (food, water) to be tested using the heterotrophic (standard) plate count method. Select dilutions that will yield plates with counts of 30-300 colonies.
2. Dispense a portion of each test dilution (e.g., 0.1 ml, 1.0 ml) into separate sterile Petri dishes.
3. Add 10-12 ml of tempered (45°C) Plate Count Agar to Petri dishes containing test dilutions.
4. Swirl the dishes to thoroughly mix the agar and test dilution.
5. Allow plates to cool and solidify.
6. Incubate at 32 ± 1°C for 48 hours.

Results

Count colonies on all plates containing 30-300 colonies. Calculate bacterial count per milliliter of sample by multiplying the average number of colonies per plate by the reciprocal of the dilution used. Report the count as CFU/ml.

References

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7. **Buchbinder, L., Y. Baris, E. Alff, E. Reynolds, E. Dillon, V. Pessin, L. Pincus, and A. Strauss.**1951. Studies to formulate new media for the standard plate count of dairy products. Pub Health Rep. **66**:327-340.
8. **Prickett, P. S.** 1928. Thermophillic and thermoduric microorganisms with special reference to species isolated from milk: V. Description of spore-forming types. Technical Bulletin. NY State Agri. Exp. Station **147**:5-58.
9. **Breed, R. S., P. A. Down, G. C. Supplee, P. S. Prickett, and G. J. Hucker.** 1932. Methods for use in the bacteriological examination of dry milk and related powders. J. Dairy Sci. **15**:383-389.

Packaging

Plate Count Agar	100 g	0479-15
	500 g	0479-17
	2 kg	0479-07
	10 kg	0479-08
Standard Methods Agar	10 x 500 ml	9081-80

Bacto® m Plate Count Broth

User Quality Control

Identity Specifications

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

Solution: 1.7% solution, soluble in distilled or deionized water; light to medium amber, clear to slightly opalescent, may have a very slight precipitate.

Reaction of 1.7% Solution at 25°C: pH 7.0 ± 0.2

Cultural Response

Prepare m Plate Count Broth per label directions. Inoculate and incubate the plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Escherichia coli	25922*	20-80	good to excellent
Staphylococcus aureus	25923*	20-80	good to excellent

The above cultures are the minimum used for performance testing.

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

Intended Use

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

Also Known As

m Plate Count Broth is also referred to as m TGY Broth, m Tryptone Glucose Yeast Broth, or m Standard Methods Broth.

Summary and Explanation

m Plate Count Broth is a nonselective general-purpose medium for determining bacterial counts from food and water samples using the membrane filtration procedure. This medium has the same formulation as Plate Count Agar except that agar has been omitted and the ingredients are employed in twice the concentration as in the solid medium.[1]

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins and amino acids. Tryptone provides carbon and nitrogen for bacterial metabolism. Dextrose is a fermentable carbohydrate and carbon source.

Formula

m Plate Count Broth

Formula Per Liter

Bacto Yeast Extract	5 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

m Plate Count Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35 ± 2°C)
Pipettes
Sterile Petri dishes, 50 x 9 mm
Membrane filter equipment
Sterile 47 mm, 0.45 µm, gridded membrane filters
Sterile absorbent pads

Method of Preparation

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

Specimen Collection and Preparation

Water samples should be collected and prepared according to recommended guidelines.[2,3,4]

Test Procedure

1. Place a sterile absorbent pad in each 50 x 9 mm Petri dish.
2. Saturate the pad with approximately 2.0-2.4 ml of prepared medium.
3. Place an inoculated membrane filter, inoculated side up, on the saturated pad.
4. Incubate in a 35 ± 2°C incubator for 18-24 hours.

Results

After incubation, count the colonies on the surface of the filter. The colonies can be subcultured to appropriate media for identification, if desired.

References

1. **MacFadden, J. F.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria. vol. 1. Williams & Wilkens, Baltimore, MD.
2. **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.
3. **Hitchins, A. D.** 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

m Plate Count Broth	100 g	0751-15
	500 g	0751-17

Bacto® Potato Dextrose Agar
Bacto Potato Dextrose Broth

Intended Use

Bacto Potato Dextrose Agar is used for culturing yeasts and molds from food and dairy products. Bacto Potato Dextrose Broth is used for cultivating yeasts and molds.

Summary and Explanation

Potato Dextrose Agar is a general purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products[1,2,3,4] and for testing cosmetics.[3] It can be used for growing clinically significant yeasts and molds.[5] The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes.[6]

Potato Dextrose Broth is a general purpose broth medium for yeasts and molds formulated as is Potato Dextrose Agar, but without agar.

Principles of the Procedure

Potato Dextrose Agar and Potato Dextrose Broth contain an infusion from potatoes and Dextrose which encourage luxuriant fungal growth. Bacto Agar is added to Potato Dextrose Agar as the solidifying agent.

Many standard procedures call for lowering the pH of Potato Dextrose Agar to 3.5 ± 0.1 to inhibit bacterial growth. The label on each container of the medium specifies the amount of sterile tartaric acid (10%) to add to the sterile medium. Do not reheat the acidified medium because heating in the acid state will hydrolyze the agar.

Formula

Potato Dextrose Agar

Formula per liter

Potatoes, Infusion from	200 g
Bacto Dextrose	20 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Potato Dextrose Broth

Formula per liter

Potatoes, Infusion from	200 g
Bacto Dextrose	20 g

Final pH 5.1 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Potato Dextrose Agar or Potato Dextrose Broth

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Sterile tartaric acid, 10% solution (optional)

Method of Preparation

Potato Dextrose Agar

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.
4. To alter the pH of the medium to 3.5 ± 0.1, add the amount of sterile 10% tartaric acid specified on the label. Do not reheat the medium after adding the acid.

Potato Dextrose Broth

1. Suspend 24 grams in 1 liter distilled or deionized water and warm slightly to dissolve completely.
2. Autoclave at 121°C for 15 minutes.

User Quality Control

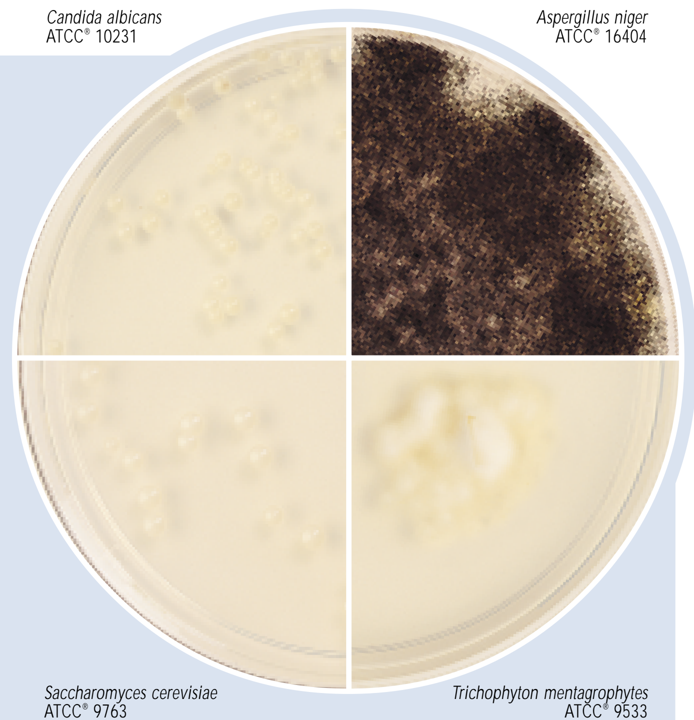
Identity Specifications

Potato Dextrose Agar

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	3.9% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 3.9% Solution at 25°C:	pH 5.6 ± 0.2

Potato Dextrose Broth

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	2.4% solution, soluble in distilled or deionized water upon slight warming; very light amber, clear.
Prepared Medium:	Very light amber, clear.
Reaction of 2.4% Solution at 25°C:	pH 5.1 ± 0.2



Cultural Response

Potato Dextrose Agar

Prepare Potato Dextrose Agar per label directions. Inoculate with test organisms. Incubate plates at 30 ± 2°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1000	good
Candida albicans	10231	100-1000	good
Saccharomyces cerevisiae	9763	100-1000	good
Trichophyton mentagrophytes	9533	undiluted	good

Potato Dextrose Broth

Prepare Potato Dextrose Broth per label directions. Inoculate medium and incubate at 30 ± 2°C for 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1000	good
Candida albicans	10231	100-1000	good
Saccharomyces cerevisiae	9763	100-1000	good

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

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Potato Dextrose Agar

Pour plate method[1,3]

1. Add 1 ml of test sample to a sterile Petri dish.
2. Add the specified amount (10 or 20 ml) of sterile, molten agar (cooled to 45- 50°C) and swirl gently to mix well. Allow to solidify.
3. Incubate at 22-25°C or 30-32°C (depending on the method being followed) for 5 days or longer.

Potato Dextrose Broth

For complete information, refer to Standard Methods procedures in the **References** section.

Results

Potato Dextrose Agar

Yeasts will grow as creamy to white colonies. Molds will grow as fuzzy colonies of various colors. Count the number of colonies and consider the dilution factor (if the test sample was diluted) in determining the yeast and/or mold counts per gram or milliliter of material.

Potato Dextrose Broth

Growth is indicated as turbidity.

Limitations of the Procedure

1. Heating Potato Dextrose Agar after acidifying hydrolyzes the agar and may destroy the solidifying properties.
2. Potato Dextrose Agar is not a differential medium. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.

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. **Frank, J. F., G. L. Christen, and L. B. Bullerman (G. H. Richardson, Tech. Comm.)** 1993. Tests for groups of microorganisms. p. 271-286. *In* **Marshall, R.T. (ed.).** Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **United States Pharmacopeial Convention**. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
5. **Dixon, D. M., and R. A. Fromtling.** 1995. Morphology, taxonomy, and classification of the fungi, p. 699-708. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1. Williams & Wilkins, Baltimore, MD.

Packaging

Potato Dextrose Agar	100 g	0013-15
	500 g	0013-17
	2 kg	0013-07
Potato Dextrose Broth	500 g	0549-17
	10 kg	0549-08

Bacto® Potato Infusion Agar

User Quality Control

Identity Specifications

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

Solution: 4.9% solution, soluble in 2% glycerol solution upon boiling. Medium amber, slightly opalescent, with a slight precipitate.

Prepared Medium: Medium amber, slightly opalescent to opalescent with a slight precipitate.

Reaction of 4.9% Solution at 25°C: pH 6.8 ± 0.2°C

Cultural Response

Prepare Potato Infusion Agar per label directions. Inoculate prepared medium and incubate at 35 ± 2°C under approximately 5-10% CO_2 for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella abortus	4315	100-1,000	good
Brucella melitensis	4309	100-1,000	good
Brucella suis	4314	100-1,000	good

Intended Use

Bacto Potato Infusion Agar is used for cultivating *Brucella*, especially in mass cultivation procedures.

Summary and Explanation

Potato Infusion Agar is prepared according to the formula used by Stockman and MacFadyean for the isolation of *Brucella abortus*. Brucellosis is a zoonotic disease with a domestic-animal reservoir.[1] Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[1]

Tryptose agar w/ 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae.[1] Most strains of *Brucella* spp. will grow on chocolate and blood agar, and the addition of 5% heated horse or rabbit serum enhances growth on all media.[2] Potato Infusion Agar permits luxuriant growth of characteristic colonies of *B. abortus* from infected materials, and may be used with excellent results in mass cultivation of *Brucella* in the preparations of vaccines and antigens.

Principles of the Procedure

Infusion from potatoes, Beef Extract and Proteose Peptone provide the nitrogen, vitamins and amino acids in Potato Infusion Agar. Dextrose and Glycerol are used as a carbon source in this formula.

Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent.

Formula

Potato Infusion Agar

Formula Per Liter

Potatoes, Infusion from	200	g
Bacto Beef Extract	5	g
Bacto Proteose Peptone	10	g
Bacto Dextrose	10	g
Sodium Chloride	5	g
Bacto Agar	15	g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. *Brucella* spp. are classified as Biosafety Level 3 pathogens. All manipulations with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).[1]

Storage

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

Expiration Date

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

Procedure

Material Provided

Potato Infusion Agar

Material Required But Not Provided

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

Method of Preparation

1. Suspend 49 grams in 1 liter distilled or deionized water containing 2% Glycerol.
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 or as desired.

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.

Test Procedure

1. Incubate plates at 35 ± 2°C in 5-10% CO_2 for 10 days.[1] For a complete discussion on the inoculation and identification of *Brucella* spp., consult appropriate references.

Results

Refer to appropriate references and procedures for results.

Limitations

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Best results are obtained on freshly prepared medium with a moist surface.

References

1. **Moyer, N. P., and L. A. Holcomb.** 1995. Brucella, p. 549-555. *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.
2. **Baron, E. J., L. R. Peterson** and **S. M. Finegold.** 1994. Bailey & Scott's Diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Potato Infusion Agar	500 g	0051-17

Bacto® Presence-Absence Broth

Intended Use

Bacto Presence-Absence Broth is used for detecting coliforms in treated water.

Also Known As

Presence-Absence Broth is abbreviated as P-A Broth.

Summary and Explanation

The Presence-Absence (P-A) test is a presumptive detection test for coliforms in water. The test is a simple modification of the multiple-tube procedure.[1] One test sample, 100 ml, is inoculated into a single culture bottle to obtain qualitative information on the presence or absence of coliforms based on the presence or absence of lactose fermentation.[1] This test is based on the principle that coliforms and other pollution indicator organisms should not be present in a 100 ml water sample.[2-8]

Comparative studies with the membrane filter procedure indicate that the P-A test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection.[1] The P-A test is described in standard methods for water testing[1] and by US EPA.[9]

Principles of the Procedure

Beef Extract, Peptone and Tryptose provides the nitrogen, vitamins and amino acids in Presence-Absence Broth. Lactose is the carbon

source in the formula. The Potassium Phosphates provide buffering capacity; Sodium Chloride maintains the osmotic balance of the medium. Sodium Lauryl Sulfate is the selective agent, inhibiting many organisms except coliforms. Brom Cresol Purple is used as an indicator dye; lactose-fermenting organisms turn the medium from purple to yellow with or without gas production.

Formula

Presence-Absence Broth (single-strength)

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Lactose	7.46 g
Bacto Tryptose	9.83 g
Potassium Phosphate, Dibasic	1.35 g
Potassium Phosphate, Monobasic	1.35 g
Sodium Chloride	2.46 g
Sodium Lauryl Sulfate	0.05 g
Brom Cresol Purple	0.0085 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Presence-Absence Broth

Materials Required But Not Provided

Glassware
Screw-cap dilution bottle with capacity > 150 ml
Incubator (35°C)

Method of Preparation

1. To prepare triple-strength medium, suspend 91.5 grams in 1 liter distilled or deionized water.
2. Warm gently to dissolve completely.
3. Dispense 50 ml amount into screw-cap 250 ml milk dilution bottles.
4. Autoclave at 121°C for 12 minutes, with the total autoclave time not to exceed 30 minutes.
5. Cool to room temperature.

Specimen Collection and Preparation

Collect water samples as described in recommended procedures.[1,9]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.05% solution, soluble in distilled or deionized water; purple, clear to very slightly opalescent without significant precipitate.
Prepared Medium:	Purple, clear to very slightly opalescent without significant precipitate.
Reaction of 3.05% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Presence-Absence Broth in triple strength solution (9.15%). Sterilize in 50 ml quantities in milk dilution bottles with capacity greater than 150 ml. Add 100 ml of drinking water after medium is sterilized and cooled to room temperature. Inoculate bottles with the test organisms. Incubate bottles for 18-48 hours at 35°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	RESULTS
Enterococcus faecalis	29212*	100-1,000	moderate	slight yellow to purple
Escherichia coli	25922*	100-1,000	good	yellow color w/ or w/o gas production
Escherichia coli	13762	100-1,000	good	yellow color w/ or w/o gas production
Pseudomonas aeruginosa	27853*	100-1,000	poor to moderate	no color change

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

Presence-Absence Broth

Test Procedure

1. Inoculate 50 ml of the sterile triple strength P-A Broth with 100 ml of the water sample.
2. Invert the bottle a few times to achieve an even distribution of the medium throughout the test sample.
3. Incubate at 35 ± 0.5°C.
4. Inspect for acid and gas production after 24 and 48 hours of incubation.

Results

A distinct yellow color indicates lactose fermentation, an acid reaction. Gas production can be observed by a foaming reaction when the bottle is gently shaken. Any amount of gas and/or acid is a positive presumptive test requiring confirmation.[1] Report results as positive or negative for coliforms per 100 ml of sample.

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 P-A test is only a presumptive test for coliforms.
3. Confirmation and differentiation of coliforms detected by the P-A test may be achieved by use of appropriate confirmatory media, incubation times and temperatures as outlined in appropriate references.[1,9]
4. Extending the P-A test incubation period to 72 or 96 hours will allow isolation of other indicator organisms. However, indicator bacteria isolated after 48 hours incubation may not be considered for regulatory purposes.

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Weiss, J. E., and C. A. Hunter.** 1939. Simplified bacteriological examination of water. J. Am. Water Works Assoc. **31**:707-713.
3. **Clark, J. A.** 1968. A presence absence (P-A) test providing sensitive and inexpensive detection of coliforms, fecal coliforms, and fecal streptococci in municipal drinking water supplies. Can. J. Microbiol. **14**:13-18.
4. **Clark, J. A.** 1969. The detection of various bacteria indicative of water pollution by a presence-absence (P-A) procedure. Can. J. Microbiol. **15**:771-780.
5. **Clark, J. A., and L. T. Vlassoff.** 1973. Relationships among pollution indicator bacteria isolated from raw water and distribution systems by the presence-absence (P-A) test. Health Lab. Sci. **10**:163-172.
6. **Clark, J. A., and J. E. Pagel.** 1977. Pollution indicator bacteria associated with municipal raw and drinking water supplies. Can. J. Microbiol. **23**:465-470.
7. **Clark, J. A.** 1980. The influence of increasing numbers of nonindicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence tests. Can. J. Microbiol. **26**:827-832.
8. **Clark, J. A., C. A. Burger, and L. E. Sabatinos.** 1982. Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. Can J. Microbiol. **28**:1002-1013.
9. **Federal Register.** 1989. National primary drinking water regulations; total coliforms (including fecal coliforms and *E. coli*). Fed regist. **54**:27544-27568.

Packaging

Presence-Absence Broth	500 g	0019-17
	2 kg	0019-07

Bacto® Proteose No. 3 Agar

Intended Use

Bacto Proteose No. 3 Agar is used with added enrichment in isolating and cultivating *Neisseria* and *Haemophilus.*

Summary and Explanation

Proteose No. 3 Agar, introduced in 1938, is used for isolating *Neisseria gonorrhoeae.* When enriched with Hemoglobin and Supplement B,[2,3] Proteose No. 3 Agar recovers gonococci in a manner comparable to more complex media, ranking only slightly lower than GC Medium at 24 hours.

Chocolate agar may be prepared from Proteose No. 3 Agar with the addition of 2% Hemoglobin. Hemoglobin provides X factor (hemin), required for growth of *Haemophilus* and enhanced growth of *Neisseria*.

The growth rate of *Neisseria* and *Haemophilus* spp. may be improved with the addition of 1% Supplement B or VX, which provide the growth factors glutamine and cocarboxylase.

Principles of the Procedure

Proteose Peptone No. 3 provides nitrogen, vitamins and amino acids. Dextrose is a carbon source. Sodium Chloride maintains the osmotic balance in the medium, which is buffered by Disodium Phosphate. Bacto Agar is the solidifying agent.

Proteose Peptone No. 3 Agar is intended for use with supplementation by 2% hemoglobin and Supplement B or Supplement VX.

Formula

Proteose No. 3 Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Bacto Dextrose	0.5 g
Sodium Chloride	5 g
Disodium Phosphate	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 Provide

Proteose No. 3 Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Hemoglobin (2%)
Supplement B or Supplement VX
Sterile Petri dishes

Method of Preparation

1. Suspend 45 grams in 500 ml liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-60°C.
4. Aseptically add 500 ml sterile 2% Hemoglobin solution. Mix well.
5. Add 10 ml of Supplement B or Supplement VX. Mix thoroughly.
6. Dispense into sterile Petri dishes or as desired.

User Quality Control

Identity Specifications

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 9% (double strength) solution, soluble in distilled or deionized water upon boiling with frequent agitation. Light to medium amber in color, opalescent with a slight flocculent precipitate.

Prepared Medium (Single-strength): Light amber, opalescent with a slight flocculent precipitate, firmly solid.

Reaction of 9% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Prepare Proteose Agar No. 3 per label directions. Inoculate and incubate at 35 ± 2°C under approximately 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Haemophilus influenzae	10211	100-1,000	good
Neisseria gonorrhoeae	43070	100-1,000	good
Neisseria meningitidis	13102	100-1,000	good
Neisseria sicca	9913*	100-1,000	good

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

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

Specimen Collection and Preparation

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 *Haemophilus* or *Neisseria* spp., refer to the procedures outlined in the references.[4,5,6]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium
2. Proteose No. 3 Agar is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.

References

1. **Carpenter, C. M., M. A. Bucca, T. C. Buck, E. P. Casman, C. W. Christensen, E. Crowe, R. Drew, J. Hill, C. E. Lankford, H. E. Morton, L. R. Peizer, C. S. Shaw, and J. D. Thayer.** 1949. Evaluation of twelve media for the isolation of the gonococcus. Am. J. Syphil. Gonorrh. Vener. Dis. **33**:164
2. **Lankford, C. E., V. Scott, M. F. Cox, and W. R. Cooke.** 1943. Some aspects of nutritional variation of the gonococcus. J. Bacteriol. **45**:321.
3. **Lankford, C. E., and E. E. Snell.** 1943. Glutamine as growth factor for certain strains of *Neisseria gonorrhoeae*. J. Bacteriol. **45**:410.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Baron, E. J., L. R. Petersons, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Proteose No. 3 Agar	500 g	0065-17
Hemoglobin 2% Solution	6 x 100 ml	3248-73
Supplement B w/Reconstituting Fluid	6 x 10 ml	0276-60
	100 ml	0276-72
Supplement VX w/Reconstituting Fluid	6 x 10 ml	3354-60
	100 ml	3354-72

Proteose Peptones

Bacto® Proteose Peptone · Bacto Proteose Peptone No. 2 Bacto Proteose Peptone No. 3

Intended Use

Bacto Proteose Peptone is used in preparing microbiological culture media and in producing bacterial toxins.

Bacto Proteose Peptone No. 2 is used in preparing microbiological culture media.

Bacto Proteose Peptone No. 3 is used in preparing microbiological culture media.

User Quality Control

Identity Specifications

Proteose Peptone

Dehydrated Appearance:	Tan, free-flowing granules.
Solution:	1%, 2% and 10% solutions are soluble in distilled or deionized water: 1%-Light amber, clear to very slightly opalescent, may have a slight precipitate; 2%-Light to medium amber, clear to slightly opalescent, may have a slight precipitate; 10%-Medium to dark amber, clear to slightly opalescent, may have a slight precipitate.
Nitrogen (Kjeldahl Method):	12.4-14.5%
Amino Nitrogen (Modified Sorensen Method):	2.0-3.75%
Reaction of 1% Solution at 25°C:	pH 6.6-7.6

Proteose Peptone No. 2

Dehydrated Appearance:	Tan, free-flowing granules.
Solution:	1%, 2% and 10% solutions are soluble in distilled or deionized water: 1%-Light to medium amber, clear, no precipitate; 2%-Medium amber, clear, no precipitate; 10%-Medium to dark amber, slightly opalescent to opalescent with precipitate.
Nitrogen (Kjeldahl Method):	11.2-12.8%
Amino Nitrogen (Modified Sorensen Method):	4.1-5.3%
Reaction of 1% Solution at 25°C:	pH 7.2-7.6

continued on following page

Summary and Explanation

Difco Laboratories conducted extensive investigations to optimize peptone production. Studies of peptic digests of animal tissue prepared under varying digestion parameters led to the development of Proteose Peptone, Proteose Peptone No. 2 and Proteose Peptone No. 3. Data accumulated during these studies demonstrated that no one peptone is the most suitable nitrogen source for every microbiological application.

Proteose Peptone was originally developed to produce a diphtheria toxin of high and uniform potency. Its suitability for this purpose was quickly established. Proteose Peptone is used in preparing toxin for diphtheria antitoxin, toxin-antitoxin mixtures, and for toxoid. Many studies support use of Proteose Peptone in culture media for diphtheria toxin production.[1,2,3,4]

Proteose Peptone is exceptionally valuable in the production of bacterial toxins, including toxins of *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Pneumococcus*, *Salmonella pullorum* and scarlet fever toxin.[5,6,7,8] Proteose Peptone has many properties that account for its suitability in culturing fastidious pathogens, including its nitrogenous components, buffering range and high proteose content. These elements create an environment suitable for the maintenance of virulence and the elaboration of bacterial by-products. For this reason, stock cultures are well preserved on media containing Proteose Peptone.

Proteose Peptone No. 2 was originally developed for use in media intended for producing diphtheria toxin. Interest was renewed by Bunney and Thomas[9] through their study of diphtheria toxin production in a semisynthetic medium. Proteose Peptone No. 2 is used in media for producing bacterial toxins and for cultivating a wide range of bacterial species.

Proteose Peptone No. 3, a modification of Proteose Peptone, is used in preparing chocolate agar for propagating *Neisseria* species and chocolate tellurite agar for propagating *Corynebacterium diphtheriae*. While investigating the nutritional values of the Proteose Peptones, Proteose Peptone No. 3 was found to provide superior nutrition for fastidious microorganisms. It can replace the meat infusion-peptone combination in infusion media. Proteose Peptone No. 3 supports growth of streptococci, staphylococci, meningococci, pneumococci, gonococci and other microorganisms requiring a highly nutritious substrate. Proteose No. 3 Agar, prepared with Proteose Peptone No. 3 as its major source of nitrogen, vitamins and amino acids, is used with added enrichments for isolating and cultivating *Neisseria* and *Haemophilus*.

Principles of the Procedure

Proteose Peptone is an enzymatic digest of protein high in proteoses. Proteose Peptone No. 2 and Proteose Peptone No. 3 are enzymatic digests of protein.

Typical Analysis

	PROTEOSE PEPTONE	PROTEOSE PEPTONE NO. 2	PROTEOSE PEPTONE NO. 3
Physical Characteristics			
Ash (%)	11.1	12.7	11.4
Clarity, 1% Solution (NTU)	1.4	1.5	2.2
Filterability (g/cm^2)	0.9	0.6	0.5
Loss on Drying (%)	3.1	3.5	4.0
pH, 1% Solution	7.2	7.2	7.2
Carbohydrate (%)			
Total	<0.1	1.3	1.4
Nitrogen Content (%)			
Total Nitrogen	14.0	12.6	13.2
Amino Nitrogen	2.9	5.0	3.5
AN/TN	20.7	39.7	26.5
Amino Acids (%)			
Alanine	6.50	6.08	5.99
Arginine	5.12	5.47	5.49
Aspartic Acid	7.28	7.45	6.92
Cystine	0.87	0.40	1.12
Glutamic Acid	11.95	10.57	12.38
Glycine	9.68	10.84	9.26
Histidine	2.01	<0.01	1.74
Isoleucine	3.04	1.00	2.65
Leucine	5.66	3.57	5.70
Lysine	5.33	5.22	5.02
Methionine	1.97	1.51	1.86
Phenylalanine	2.86	7.94	2.72
Proline	5.93	5.31	4.94
Serine	3.49	4.64	3.65
Threonine	3.14	3.90	3.32
Tryptophan	0.60	0.94	0.59
Tyrosine	2.35	1.92	1.96
Valine	3.76	4.73	3.62

User Quality Control cont.

Proteose Peptone No. 3

Dehydrated Appearance: Golden tan, free-flowing granules.

Solution: 1%, 2% and 10% solutions are soluble in distilled or deionized water:
1%-Very light amber, clear to very slightly opalescent, may have a slight precipitate;
2%-Light amber, clear to slightly opalescent, may have a slight precipitate;
10%-Light to medium amber, clear to slightly opalescent, may have a slight precipitate.

Nitrogen (Kjeldahl Method): 11.5-13.3%

Amino Nitrogen (Modified Sorensen Method): 2.25-4.85%

Reaction of 1% Solution at 25°C: pH 7.0-7.6

continued on following page

	PROTEOSE PEPTONE	PROTEOSE PEPTONE NO. 2	PROTEOSE PEPTONE NO. 3
Inorganics (%)			
Calcium	0.021	0.024	0.023
Chloride	4.510	3.644	3.581
Cobalt	<0.001	<0.001	<0.001
Copper	<0.001	<0.001	<0.001
Iron	0.002	<0.001	0.002
Lead	<0.001	<0.001	<0.001
Magnesium	0.027	0.024	0.027
Manganese	<0.001	<0.001	<0.001
Phosphate	0.872	1.674	1.447
Potassium	0.685	0.815	0.982
Sodium	3.677	3.956	3.815
Sulfate	0.162	0.232	0.232
Sulfur	0.812	0.698	0.975
Tin	<0.001	<0.001	<0.001
Zinc	0.002	0.003	0.007
Vitamins (µg/g)			
Biotin	0.1	0.3	0.4
Choline (as Choline Chloride)	2300.0	4500.0	3700.0
Cyanocobalamin	<0.1	<0.1	<0.1
Folic Acid	0.4	0.5	0.3
Inositol	5000.0	4700.0	8900.0
Nicotinic Acid	79.9	157.1	124.2
PABA	4.2	1.2	<0.5
Pantothenic Acid	20.0	47.0	20.0
Pyridoxine	1.1	4.0	1.3
Riboflavin	<0.1	6.4	6.8
Thiamine	1.2	1.6	0.1
Thymidine	99.7	1319.0	659.6
Biological Testing (CFU/g)			
Coliform	negative	negative	negative
Salmonella	negative	negative	negative
Spore Count	393	75	890
Standard Plate Count	443	1450	915
Thermophile Count	73	<50	25

The values presented above 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.

Precautions

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

Storage

Store 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

Proteose Peptone
Proteose Peptone No. 2
Proteose Peptone No. 3
H_2S Test Strips
Indole Test Strips
KL Antitoxin Strips
KL Virulence Enrichment

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Proteose Peptone, Proteose Peptone No. 2 or Proteose Peptone No. 3 in the formula of the medium being prepared. Add as required.

Specimen Collection and Preparation

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

Test Procedure

See appropriate references for specific procedures using Proteose Peptone, Proteose Peptone No. 2 or Proteose Peptone No. 3.

Results

Refer to appropriate references and procedures for results.

References

1. **Hewitt.** 1930. Biochem. J. **24**:984.
2. **Bunney.** 1930. J. Immunol. **20**:71.
3. **Kirkbride, Berthelsen and Clark.** 1931. J. Immunol. **21**:1.
4. **Hazen and Heller.** 1932. J. Bacteriol. **23**:195.
5. **Kirkbride and Wheeler.** 1926. J. Immunol. **11**:477.
6. **Nelson.** 1927. J. Infect. Dis. **41**:9.
7. **Kneeland and Dawes.** 1932. J. Exp. Med. **55**:735.
8. **Hanks and Rettger.** 1932. J. Immunol. **22**:283.
9. **Bunney and Thomas.** 1936. J. Immunol. **31**:95.

Packaging

Proteose Peptone	500 g	0120-17
	10 kg	0120-08
Proteose Peptone No. 2	500 g	0121-17
	10 kg	0121-08
Proteose Peptone No. 3	500 g	0122-17
	2 kg	0122-07
	10 kg	0122-08

User Quality Control cont.

Cultural Response

Proteose Peptone, Proteose Peptone No. 2 and Proteose Peptone No. 3

For each Test specified, prepare a Test Solution of the desired Proteose Peptone 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 Carbohydrate	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 (AMC)	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 Response	2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose	*Brucella suis*	4314	undiluted	good growth
Growth Response	2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose	*Staphylococcus aureus*	25923*	100-1,000 CFU	good growth
Growth Response	2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose	*Escherichia coli*	25922*	100-1,000 CFU	good growth

Proteose Peptone

Prepare KL Virulence Agar from individual ingredients using 2 grams of the test Proteose Peptone; sterilize, add KL Virulence Enrichment and dispense into Petri dishes containing KL Antitoxin Strips. Inoculate with a loopful of surface growth and incubate at 35 ± 2°C for 72 hours. Examine at 24, 48 and 72 hours.

TEST	ORGANISM	ATCC®	RESULT
Toxin Production	*Corynebacterium diphtheriae* Type intermedius	8032	precipitin line
Toxin Production	*Corynebacterium diphtheriae* Type gravis	8028	precipitin line
Toxin Production	*Corynebacterium diphtheriae* Type mitis	8024	precipitin line

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.

Pseudomonas Agar Media
Bacto® Pseudomonas Agar F · Bacto Pseudomonas Agar P

Intended Use

Pseudomonas Agar F is used with Bacto Glycerol for detecting and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on fluorescein production.

Pseudomonas Agar P is used with Bacto Glycerol for detecting and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on pyocyanin production.

Also Known As

Pseudomonas Agar F is known as Pseudomonas Agar Medium for Detection of Fluorescein.

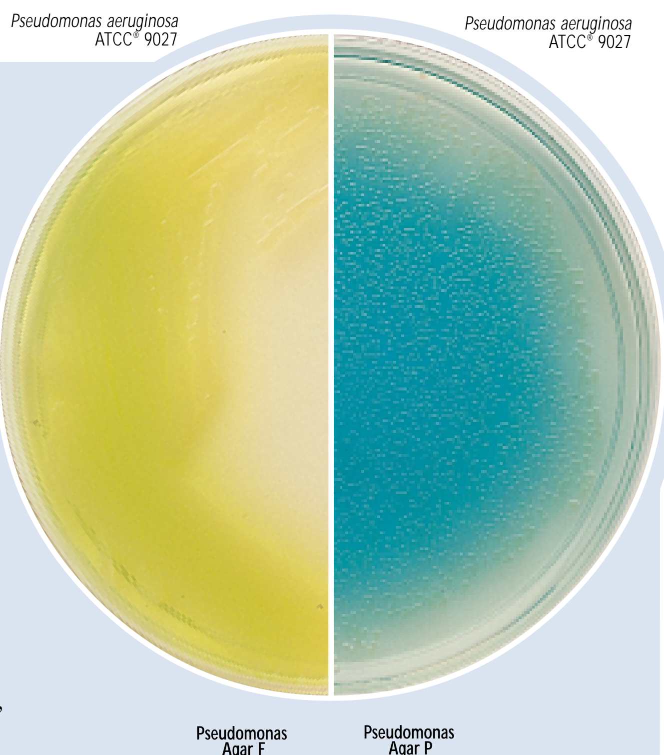
Pseudomonas Agar P is also known as Pseudomonas Agar Medium for Detection of Pyocyanin.

Summary and Explanation

Pseudomonas Agar F and Pseudomonas Agar P, patterned after the formulations described by King, Ward and Raney,[1] are modified to USP specifications.[2]

Pseudomonas Agar F enhances the production of fluorescein by *Pseudomonas* and inhibits the formation of pyocyanin. Pseudomonas Agar P, in contrast, enhances the production of pyocyanin and inhibits the formation of fluorescein. Both pigments diffuse from *Pseudomonas* colonies into the medium in which they grow. Fluorescein elaborated on Pseudomonas Agar F is a fluorescent yellow color, while pyocyanin elaborated on Pseudomonas Agar P is a blue color.

Some *Pseudomonas* strains elaborate both pigments, while others



User Quality Control

Identity Specifications

Pseudomonas Agar F

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

Solution: 3.8% solution with 1% Glycerol, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.

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

Reaction of 3.8% Solution at 25°C: pH 7.0 ± 0.2

Pseudomonas Agar P

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

Solution: 4.64% solution with 1% Glycerol, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.

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

Reaction of 4.64% Solution at 25°C: pH 7.0 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	GROWTH	PIGMENT PRODUCTION PSEUDOMONAS AGAR F	PIGMENT PRODUCTION PSEUDOMONAS AGAR P
Pseudomonas aeruginosa	9027	good	greenish yellow	blue
Pseudomonas aeruginosa	27853*	good	greenish yellow	blue
Pseudomonas cepacia	25609	good	no pigment	no pigment

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

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

elaborate only one of the two. When Pseudomonas Agar F and Pseudomonas Agar P are used together, they provide for easy and rapid identification of most *Pseudomonas* strains as specified in the FDA Bacteriological Analytical Manual.[3]

Principles of the Procedure

Pseudomonas Agar F

Tryptone and Proteose Peptone No. 3 provide carbon and nitrogen sources required for good growth and also aid in fluorescein production. Phosphate stimulates fluorescein production and has an inhibitory effect on pyocyanin. Dipotassium Phosphate increases the phosphorus content over that supplied by the peptones. Magnesium Sulfate provides necessary cations for the activation of fluorescein production. Bacto Agar is a solidifying agent. Glycerol, added during preparation of the medium, is a carbon source.

Pseudomonas Agar P

Bacto Peptone provides the carbon and nitrogen sources required for good growth. Glycerol is a carbon source. Magnesium Chloride and Potassium Sulfate stimulate pyocyanin production. Bacto Agar is a solidifying agent.

Formula

Pseudomonas Agar F

Formula Per Liter

Ingredient	Amount
Bacto Tryptone	10 g
Bacto Proteose Peptone No. 3	10 g
Dipotassium Phosphate	1.5 g
Magnesium Sulfate	1.5 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Pseudomonas Agar P

Formula Per Liter

Ingredient	Amount
Bacto Peptone	20 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory 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 media at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Pseudomonas Agar F
Pseudomonas Agar P

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Sterile Petri dishes
Tubes with closures
Bacto Glycerol

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water containing 10 grams of Glycerol:
   Pseudomonas Agar F - 38 grams;
   Pseudomonas Agar P - 46.4 grams.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Obtain the inoculum from a pure 18-24 hour culture of *Pseudomonas*.
2. Inoculate plates or agar slants by streaking the surface.
3. Incubate at 35 ± 2°C for 18-24 hours.

Results

Examine colonies under ultraviolet light (Wood's lamp).[4] Take care when using UV illumination because it may have a bactericidal effect. Be sure there is good growth before placing the culture under UV light.

Pseudomonas Agar F: Positive result is indicated by a light, bright greenish-yellow color diffusing into the agar with a fluorescent zone surrounding the growth.

Pseudomonas Agar P: Positive result is indicated by a blue pigment that diffuses into the agar.

Limitations of the Procedure

1. Occasionally, a *Pseudomonas* culture is encountered that will produce small amounts of pigment in the medium. When this happens, a yellow-green color will appear on Pseudomonas Agar F or a blue-green color on Pseudomonas Agar P. If a blue-green color occurs on Pseudomonas Agar P, confirmation of the presence of pyocyanin can be made by extraction with chloroform ($CHCl_3$).[4]
2. The formation of nonpigmented colonies does not completely rule out a *Pseudomonas aeruginosa* isolate.
3. A pyocyanin-producing *Pseudomonas* strain will usually also produce fluorescein. It must, therefore, be differentiated from other simple fluorescent pseudomonads by other means. Temperature can be a determining factor as most other fluorescent strains will not grow at 35°C. Rather, they grow at 25-30°C.[4]

References

1. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301.
2. **The United States Pharmacopeia.** 1995. The United States pharmacopeia, 23rd ed. United States Pharmacopeial Convention, Rockville, MD.

3. **Bacteriological Analytical Manual, 8th edition.** 1995. AOAC International, Gaithersburg, MD.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Pseudomonas Agar F	100 g	0448-15
	500 g	0448-17
Pseudomonas Agar P	500 g	0449-17

Bacto® Pseudomonas Isolation Agar

Intended Use

Bacto Pseudomonas Isolation Agar is used with added glycerol in isolating *Pseudomonas* and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on pigment formation.

Summary and Explanation

Pseudomonas aeruginosa is an opportunistic pathogen that can infect eyes, ears, burns and wounds.[2,4] It is also a leading cause of hospital acquired infections. Patients undergoing antibiotic therapy are especially susceptible to infection by *Pseudomonas aeruginosa*.

Pseudomonas Isolation Agar is prepared according to a slight modification of the Medium A formulation of King, Ward and Raney.[1] It is especially useful for isolating *Pseudomonas* from clinical specimens such as stools, wounds and urine.[2] Pseudomonas Isolation Agar includes Irgasan®, a potent broad spectrum antimicrobial that is not active against *Pseudomonas*.[3] As well as being selective, Pseudomonas Isolation Agar is formulated to enhance the formation of the blue or blue-green pyocyanin pigment by *Pseudomonas aeruginosa*. The pigment diffuses into the medium surrounding growth.

Principles of the Procedure

Bacto Peptone provides the carbon and nitrogen necessary for bacterial growth. Magnesium Chloride and Potassium Sulfate promote production of pyocyanin. Irgasan, an antimicrobial agent, selectively inhibits gram-positive and gram-negative bacteria other than *Pseudomonas* spp. Bacto Agar is a solidifying agent. Glycerol serves as an energy source and also helps to promote pyocyanin production.

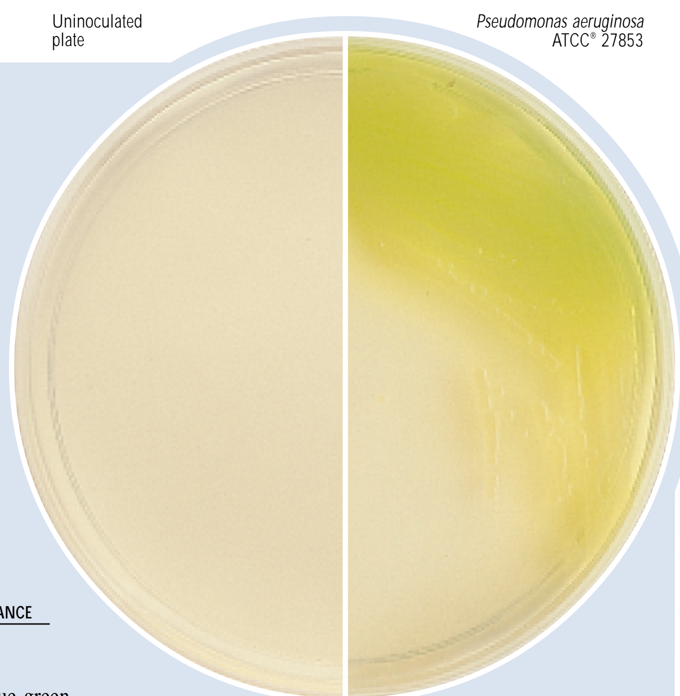
Formula

Pseudomonas Isolation Agar

Formula Per Liter

Bacto Peptone	20 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10 g
Irgasan®	0.025 g
Bacto Agar	13.6 g

Final pH 7.0 ± 0.2 at 25°C



User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige, homogeneous, free-flowing.
Solution:	4.5% solution, soluble on boiling in distilled or deionized water containing 2% glycerol. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent, firm.
Reaction of 4.5% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	
Pseudomonas aeruginosa	10145	100-1,000	good	green to blue-green
Pseudomonas aeruginosa	27853*	100-1,000	good	green to blue-green

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

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

Pseudomonas Isolation Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 45 grams in 980 ml distilled or deionized water.
2. Add 20 ml of Glycerol.
3. Boil to dissolve completely.
4. Autoclave at 121°C for 15 minutes.

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.[2,4,5]
2. Process each specimen, using procedures appropriate for that specimen or sample.[2,4,5]

Test Procedure

1. Inoculate the medium using the streak plate method to obtain isolated colonies.
2. Incubate for 18-48 hours at 35 ± 2°C.

Results

Examine for the presence of good growth. *Pseudomonas aeruginosa* colonies will be green to blue-green with pigment that diffuses into the medium.

Limitations of the Procedure

1. Some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.[6]
2. Non-*Pseudomonas aeruginosa* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Pseudomonas aeruginosa*. Consult appropriate references.[2,5]

References

1. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. & Clin. Med. **44(2)**:301-307.
2. **Baron, E. J., and S. M. Finegold.** 1990. Bailey & Scott's Diagnostic Microbiology, 8th ed. C.V. Mosby Company, St. Louis, MO.
3. **Furia and Schenkel.** 1968. Soap and Chemical specialties. January.
4. **Gilligan, P. H.** 1995. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
5. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.0-1.20.47. *In* H. D. Isenberg, (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. **Gaby, W. L., and E. Free.** 1931. J. Bacteriol. **22:**349.

Packaging

Pseudomonas Isolation Agar	500 g	0927-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Purple Broth Base
Bacto Purple Agar Base

Intended Use

Bacto Purple Broth Base and Purple Agar Base are used with added carbohydrate in differentiating pure cultures of bacteria, particularly of enteric organisms, based on fermentation reactions.

Summary and Explanation

Purple Broth Base and Purple Agar Base are carbohydrate-free fermentation media that are preferred by some bacteriologists because of their slightly acid reaction (pH 6.8). When supplemented with carbohydrates, these media are useful in obtaining accurate fermentation reactions in the identification of *Enterobacteriaceae* and other microorganisms. The concentration of carbohydrate generally employed for testing the fermentation reactions of bacteria is 0.5 or 1%. Some investigators prefer to use 1% rather than 0.5% to insure against reversion of the reaction due to depletion of the carbohydrate by some microorganisms. Purple Broth Base with added carbohydrates is specified in several standard methods.[1,2,3,4]

Principles of the Procedure

Proteose Peptone No. 3 and Beef Extract provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Sodium Chloride maintains the osmotic balance of the medium. Brom Cresol Purple serves as an indicator, assuming a yellow color when

acid is produced during the fermentation of the added carbohydrate. In Purple Agar Base, the Bacto Agar serves as a solidifying agent.

Formula

Purple Broth Base

Formulas Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Sodium Chloride	5 g
Bacto Brom Cresol Purple	0.02 g

Final pH at 25°C 6.8 ± 0.2

User Quality Control

Identity Specifications

Purple Broth Base

Dehydrated Appearance:	Light tan with grayish-green cast, free-flowing, homogeneous.
Solution:	1.6% solution soluble in distilled or deionized water. Solution is purple, clear to very slightly opalescent.
Prepared Tubes:	Purple, clear to very slightly opalescent.
Reaction of 1.6% Solution at 25°C:	pH 6.8 ± 0.2

Purple Agar Base

Dehydrated Appearance:	Light tan with grayish-green cast, free-flowing, homogeneous.
Solution:	3.1% solution soluble in distilled or deionized water upon boiling. Solution is purple, very slightly to slightly opalescent.
Prepared Medium:	Purple, slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Purple Broth Base

Prepare Purple Broth Base per label directions with 1.0% Dextrose. Inoculate and incubate the tubes at 35 ± 2°C for 18-48 hours. A color change to yellow indicates acid production, and the appearance of bubbles in the inverted fermentation vial indicates gas production.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	REACTION w/1% DEXTROSE ACID	GAS
Alcaligenes faecalis	8750	100-1,000	good	–	–
Escherichia coli	25922*	100-1,000	good	+	+
Salmonella typhimurium	14028*	100-1,000	good	+	+

Purple Agar Base

Prepare Purple Agar Base per label directions with 1.0% Dextrose. Inoculate tubes with test organisms by stabbing the butt of the tube and streaking the slant. Incubate at 35 ± 2°C for 18-48 hours. A color change to yellow indicates acid production, and the appearance of bubbles indicates gas production.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	REACTION w/1% DEXTROSE ACID	GAS
Alcaligenes faecalis	8750	1,000-2,000	good	–	–
Escherichia coli	25922*	1,000-2,000	good	+	+
Salmonella typhimurium	14028*	1,000-2,000	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 — Typical positive growth with acid and gas reaction — Typical negative growth with acid and gas reaction

Uninoculated tube — *Escherichia coli* ATCC® 25922 — *Escherichia coli* ATCC® 25922 + Dextrose

Purple Agar Base

Purple Agar Base

Formulas Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Sodium Chloride	5 g
Bacto Brom Cresol Purple	0.02 g
Bacto Agar	15 g

Final pH at 25°C 6.8 ± 0.2

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

Procedure

Materials Provided

Bacto Purple Broth Base
Bacto Purple Agar Base

Materials Required But Not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Choice of carbohydrates
Fermentation vials (Purple Broth Base)

Method of Preparation

Purple Broth Base

1. Suspend 16 grams in 1 liter distilled or deionized water and heat to boiling to dissolve completely.
2. Autoclave at 121°C for 15 minutes.

   To prepare fermentation broths, add 0.5-1% carbohydrate before or after sterilization, depending on heat lability. Dispense into tubes containing inverted fermentation vials.

Purple Agar Base

1. Suspend 31 grams in 1 liter distilled or deionized water and boil to dissolve completely.
2. To prepare 0.5-1% carbohydrate fermentation agars, dissolve 5-10 grams of the desired carbohydrate in the basal medium prior to sterilization.
3. Autoclave at 121°C for 15 minutes.

OR

1. Dissolve 31 grams in 900 ml distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes.
3. Cool the basal medium to 45-50°C
4. Aseptically add 100 ml sterile 5-10% carbohydrate solution (w/v).

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate tubes using a light inoculum from an 18-24 hour pure culture. To inoculate Purple Broth Base tubes, use a loopful of inoculum. For Purple Agar Base tubes, stab with an inoculating needle to within 1/4 inch from the bottom of the tube.
2. Incubate tubes for 24-72 hours at 35 ± 2°C in an aerobic or anaerobic atmosphere, depending on the organisms being tested.
3. Examine tubes daily for acid production and gas formation. Hold negative tubes for a total of 30 days.

Results

A yellow color is a positive reaction for fermentation of the carbohydrate. Bubbles in the inverted fermentation vials are an indication of gas production. Even the presence of a single bubble is significant to record as positive.[5]

Limitations of the Procedure

1. The addition of some carbohydrates to the media may result in an acid reaction. In this case, it is suggested that the proper pH be restored by adding sterile 0.1N sodium hydroxide dropwise.
2. Avoid excessive heating or prolonged heat exposure of media to avoid hydrolysis of the carbohydrates.
3. Tubes should be tightly stoppered during the incubation period for fermentation studies of the enteric group to avoid reversion caused by rapid depletion of the carbohydrate(s).[5]

References

1. **Bacteriological Analytical Manual,** 8th edition. 1995. AOAC International, Gaithersburg, MD.
2. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products. American Public Health Assoc., Washington, D.C.
3. **Vanderzant, C., and D. F. Splittstoesser.** 1992. Compendium of methods for the microbiological examination of foods. American Public Health Assoc., Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995 Official methods of analysis of AOAC International. AOAC International, Arlington, VA.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Purple Broth Base	500 g	0227-17
Purple Agar Base	500 g	0228-17

Bacto® Purple Lactose Agar

Intended Use

Bacto Purple Lactose Agar is used for cultivating coliform organisms; for differentiating lactose-fermenting from lactose-nonfermenting organisms.

Summary and Explanation

Purple Lactose Agar is a modification of Litmus Lactose Agar, described by Wurtz.[1] In Purple Lactose Agar, brom cresol purple replaces litmus, which is less selective and less stable.

Purple Lactose Agar is used for detecting coliforms and in differential studies based on the fermentation of lactose. Tests used to differentiate *Enterobacteriaceae* determine the organism's ability to use a carbohydrate with the production of acid metabolic end products.[2] Colonies of lactose-fermenting organisms are differentiated from lactose non-fermenters by a color change of the indicator from blue-purple (alkaline) to yellow (acid). If gas is produced during fermentation of the carbohydrate, bubbles will appear in the medium.[2]

Principles of the Procedure

Beef Extract and Bacto Peptone provide the nitrogen, vitamins and amino acids in Purple Lactose Agar. Bacto Lactose is the carbohydrate used in the fermentation reaction. Bacto Agar is the solidifying agent. Bacto Brom Cresol Purple is the pH indicator.

Formula

Purple Lactose Agar

Formula Per Liter

Bacto Beef Extract	3	g
Bacto Peptone	5	g
Bacto Lactose	10	g
Bacto Agar	10	g
Bacto Brom Cresol Purple	0.025	g

Final pH 6.8 ± 0.1 at 25°C

Precautions

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

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

Purple Lactose Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator

User Quality Control

Identity Specifications

Dehydrated Appearance: Light beige with greenish cast, free-flowing and homogeneous.

2.8% Solution: Soluble in distilled or deionized water on boiling. Solution is purple, clear to very slightly opalescent.

Reaction of 2.8% Solution: pH 6.8 ± 0.1 at 25°C

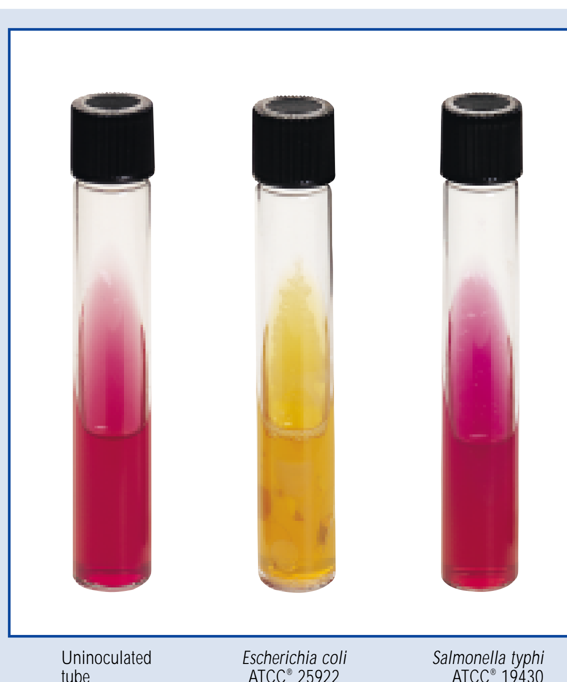
Cultural Response

Inoculate the agar slant by stabbing the butt and streaking with an inoculating needle. Incubate tubes at 35 ± 2°C for 18-48 hours. Acid production is indicated by a yellow color.

ORGANISM	ATCC®	GROWTH	ACID (YELLOW)	GAS
Enterobacter aerogenes	13048*	good	+	+
Escherichia coli	25922*	good	+	+
Salmonella typhi	19430	good	–	–
Staphylococcus aureus	25923*	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 — *Salmonella typhi* ATCC® 19430

Method of Preparation

1. Suspend 28 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 tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For a complete discussion on the expected reactions of specific Enterobacteriaceae species, refer to Manual of Clinical Microbiology,[3] Clinical Microbiology Procedures Handbook[4] and Bailey & Scott's Diagnostic Microbiology.[2]

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. Medium is slightly acid (pH 6.8) and positive reactions may be slower than with phenol red carbohydrate medium.[5]

References

1. **Wurtz.** 1897. Technique Bacteriologique, Masson, Paris.
2. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1. Williams & Wilkins, Baltimore, MD.

Packaging

Purple Lactose Agar	500 g	0082-17

Bacto® Pyridoxine Y Medium

Intended Use

Bacto Pyridoxine Y Medium is used for determining pyridoxine 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.

Pyridoxine Y Medium is patterned after the formulation of Campling and Nixon,[1] and modified by Hurley[2] and Parrish, Loy and Kline.[3] This medium is used in the microbiological assay of pyridoxine using *Saccharomyces cerevisiae* ATCC® 9080 (*Saccharomyces uvarum)* as the test organism.

User Quality Control

Identity Specifications

Dehydrated Appearance:	White to off-white, fine, free-flowing, homogeneous.
Solution:	2.65% (single strength) 5.3% (double strength) solution, soluble in distilled or deionized water upon boiling for 2-3 minutes. Solution is almost colorless to very light amber, clear, may have a slight precipitate.
Prepared Medium:	Single strength solution is colorless to very light amber, clear, may have a slight precipitate.
Reaction of 2.65% Solution at 25°C:	pH 4.4 ± 0.2

Cultural Response

Prepare Pyridoxine Y Medium per label directions. This medium should support the growth of *Saccharomyces cerevisiae* ATCC® 9080 when prepared in single strength and supplemented with a mixture containing 1 ng per ml each of pyridoxal hydrochloride, pyridoxamine hydrochloride and pyridoxine hydrochloride.

Principles of the Procedure

Pyridoxine Y Medium is free from pyridoxine, but contains all other nutrients and vitamins essential for the growth of *S. cerevisiae* ATCC® 9080. The addition of pyridoxine in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Pyridoxine Y Medium

Formula Per Liter

Ingredient	Amount
L-Asparagine	4 g
L-Histidine Hydrochloride	20 mg
DL-Methionine	40 mg
DL-Tryptophane	40 mg
DL-Isoleucine	40 mg
DL-Valine	40 mg
Bacto Dextrose	40 g
Thiamine Hydrochloride	400 µg
Calcium Pantothenate	400 µg
Nicotinic Acid	400 µg
Biotin Salt	8 mg

Riboflavin	20	µg
Inositol	5	mg
Boric Acid	200	µg
Monopotassium Phosphate	3	g
Magnesium Sulfate	1	g
Ammonium Sulfate	4	g
Calcium Chloride	0.49	g
Potassium Iodide	200	µg
Ammonium Molybdate	40	µg
Manganese Sulfate	80	µg
Copper Sulfate	90	µg
Zinc Sulfate	80	µg
Ferrous Sulfate	500	µg

Final pH 4.4 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Pyridoxine Y Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Saccharomyces cerevisiae* ATCC® 9080
Sterile tubes
Distilled or deionized water
Pyridoxal HCl
Pyridoxamine 2 HCl
Pyridoxine HCl
Lactobacilli Agar AOAC
Shaker (100 rpm)
Incubator (25-30°C)
Centrifuge
Ethyl alcohol
Spectrophotometer

Method of Preparation

1. Dissolve 5.3 grams in 100 ml distilled or deionized water.
2. Boil 2- 3 minutes to dissolve completely.
3. Dispense 5 ml amounts into flasks, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust flask volume to 10 ml with distilled or deionized water.
6. Steam at 100°C for 10 minutes.

Specimen Collection and Preparation

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

Test Procedure

Stock cultures of *S. cerevisiae* ATCC® 9080 are carried on Lactobacilli Agar AOAC. Following incubation at 25-30°C (held constant within ±0.5°C) for 18-24 hours, store the cultures in the dark at 2-8°C. Prepare fresh slant cultures every week. Do not use stock cultures for preparing the inoculum if more than one week old. Inoculum for assay is prepared by subculturing a stock culture of *S. cerevisiae* ATCC 9080 into a tube (10 ml) of single strength Pyridoxine Y Medium containing 1 ng per ml each of pyridoxal hydrochloride, pyridoxamine dihydrochloride and pyridoxine hydrochloride. After 18-24 hours incubation at 25-30°C (held constant within ±0.5°C), centrifuge the cells under aseptic conditions and decant the liquid supernatant. Wash the cells 3x with 10 ml sterile 0.85% saline. After the third wash, resuspend in 10 ml sterile single strength medium and adjust to a turbidity of 45-50% transmittance when read on the spectrophotometer at 660 nm.

It is essential that a standard curve be set up for each separate assay. Conditions of steaming and temperature of incubation which influence the standard curve readings cannot always be duplicated. Obtain the standard curve by using pyridoxine hydrochloride at levels of 0, 1, 2, 4, 6, 8 and 10 ng per flask (10 ml).

The concentrations of pyridoxine hydrochloride required for the preparation of the standard curve may be prepared as follows:

A. Dissolve 50 mg dried pyridoxine hydrochloride in about 100 ml in HCL solution.

B. Dilute 500 ml with in HCL.

C. Further dilute by adding 2 ml to 998 ml distilled water to make a stock solution containing 200 ng pyridoxine hydrochloride per ml. Prepare the stock solution fresh daily.

To make the standard solution, dilute 1 ml of stock solution with 99 ml distilled water, to make a solution containing 2 ng pyridoxine hydrochloride per ml. Use 0.0, 0.05, 1, 2, 3, 4 and 5 ml per assay tube.

Following inoculation, incubate the tubes on a shaker (about 100 rpm) at 25-30°C for 22 hours. Steam in the autoclave for 5 minutes to stop growth. Measure the growth turbidimetrically using a spectrophotometer at any specific wavelength between 540 and 660 nm.

Results

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

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be grown and maintained on a medium 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. **Campling, and Nixon.** 1954. J. Physiol. **126**:71.
2. **Hurley.** 1960. J. AOAC. **43**:43.
3. **Parrish, Loy, and Kline.** 1956. J. AOAC. **39**:157.

Packaging

Pyridoxine Y Medium	100 g	0951-15*

*Store at 2-8°C

Bacto® R2A Agar

Intended Use

Bacto R2A Agar is used for enumerating heterotrophic organisms in treated potable water.

Summary and Explanation

R2A Agar was developed by Reasoner and Geldreich[1] for bacteriological plate counts of treated potable water. A low nutrient medium, such as R2A Agar, in combination with a lower incubation temperature and longer incubation time stimulates the growth of stressed and chlorine-tolerant bacteria.[1] Nutritionally rich media, such as Tryptone Glucose Yeast Extract Agar (TGEA) or Plate Count Agar (PCA), support the growth of fast-growing bacteria but may suppress slow growing or stressed bacteria found in treated water. When compared with TGEA and PCA, R2A Agar has been reported to improve the recovery of stressed and chlorine-tolerant bacteria from drinking water systems.[2,3,4]

R2A Agar is recommended in *Standard Methods for the Examination of Water and Wastewater*[5] for pour plate, spread plate and membrane filter methods for heterotrophic plate counts.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.82% solution, soluble in distilled or deionized water on boiling. Solution is light amber in color, slightly opalescent, with a slight precipitate.
Prepared Plates:	Light amber in color, slightly opalescent, with a slight precipitate.
Reaction of 1.82% Solution at 25°C:	7.2 ± 0.2.

Cultural Response

Prepare R2A Agar per label directions. Inoculate with tap water samples using the streak plate method and/or the membrane filter method. Incubate at 35 ± 2°C for 40-72 hours. Recovery is typical compared to an approved control lot and greater than parallel plates of Plate Count Agar.

Principles of the Procedure

Yeast Extract provides a source of trace elements and vitamins. Proteose Peptone No. 3 and Casamino Acids provide nitrogen, vitamins, amino acids, carbon and minerals. Dextrose serves as a carbon source. Soluble Starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Sodium Pyruvate increases the recovery of stressed cells. Potassium Phosphate is used to balance the pH and provide phosphate. Magnesium Sulfate is a source of divalent cations and sulfate. Bacto Agar is the solidifying agent.

Formula

R2A Agar

Formula Per Liter

Bacto Yeast Extract	0.5 g
Bacto Proteose Peptone No. 3	0.5 g
Bacto Casamino Acids	0.5 g
Bacto Dextrose	0.5 g
Soluble Starch	0.5 g
Sodium Pyruvate	0.3 g
Potassium Phosphate, Dibasic	0.3 g
Magnesium Sulfate	0.05 g
Bacto Agar	15 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

R2A Agar

Materials Required But Not Provided

Autoclave
Petri dishes
Membrane filter equipment and filters
Dilution blanks
Pipettes or glass rods
Incubator (20, 28 or 35°C)
Colony counter

Method of Preparation

1. Suspend 18.2 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

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

To minimize changes in bacterial population, water samples should be tested as soon as possible, but at least within six hours of collection if the sample has not been refrigerated or within 30 hours if refrigerated.

Test Procedure

1. Prepare test dilutions for heterotrophic plate count.
2. Plate the test sample and dilutions by the spread plate, pour plate or membrane filter method. Do not exceed 1 ml of sample or dilution per spread or pour plate. The volume of test sample to be filtered for the membrane filter technique will vary.
3. Maintain proper humidity during prolonged incubation:

INCUBATION TEMPERATURE	MINIMUM INCUBATION TIME[3]	OPTIMAL INCUBATION TIME[3]
35°C	72 hours	5-7 days
20 or 28°C	5 days	7 days

Results

Count colonies on spread or pour plates demonstrating 30-300 colonies per plate or 20-200 colonies when using the membrane filter method. Compute bacterial count per ml of sample by multiplying the average number of colonies per plate by the reciprocal of the appropriate dilution.

Report counts as colony forming units (CFU) per ml and report variables of incubation such as temperature and length of time.

Limitations of the Procedure

1. R2A Agar is intended for use only with treated potable water since it is recommended for compromised bacteria.
2. Use of the pour plate method is discouraged because recovery of stressed bacteria may be compromised by the heat shock (44-46°C) and low oxygen tension that are part of the procedure.[6,7]
3. Incubation time longer than indicated above may be necessary to recover additional slow-growing bacteria.
4. R2A Agar performs best with the spread plate technique; however, that procedure is limited to a small sample volume.
5. Fast-growing bacteria may produce smaller size colonies on R2A Agar than on nutritionally rich media.
6. R2A Agar is a low nutrient medium intended for culturing compromised microorganisms. Good growth of standard, healthy control organisms does not necessarily reflect the ability of the medium to recover stressed organisms. Each new lot of medium should be performance tested against a previous lot of R2A Agar using tap water.

References

1. **Reasoner, D. J., and E. E. Geldreich.** 1979. A new medium for the enumeration and subculture of bacteria from potable water. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.
2. **Fiksdal, L., E. A. Vik, A. Mills, and T. Staley**. 1982. Non-standard methods for enumerating bacteria in drinking water. Journal AWWA. **74**:313-318.
3. **Kelly, A. J., C. A. Justice, and L. A. Nagy**. 1983. Predominance of chlorine tolerant bacteria in drinking water systems. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. Q122.
4. **Means, E. G., L. Hanami, H. F. Ridgway, and B. H. Olson**. 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. Journal AWWA. **53**:585-590.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.)**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Van Soestberger, A. A., and C. H. Lee**. 1969. Pour plates or streak plates? Appl. Microbiol. **18**:1092.
7. **Klein, D. A., and S. Wu**. 1974. Stress: a factor to be considered in heterotrophic microorganism enumeration from aquatic environments. Appl. Microbiol. **27**:429.

Packaging

R2A Agar	100 g	1826-15
	500 g	1826-17
	2 kg	1826-07

Bacto® Raka-Ray No. 3 Broth Bacto Raka-Ray No. 3 Medium

Intended Use

Bacto Raka-Ray No. 3 Broth and Medium are recommended for the isolation of lactic acid bacteria encountered in beer and the brewing process.

Summary and Explanation

Spoilage organisms are often seriously detrimental to beer flavor. Lactic acid bacteria including lactobacilli and pediococci which can cause spoilage are physiologically very diverse..

Raka-Ray No. 3 Broth and Medium were developed from a formulation suggested by Saha, Sondag, and Middlekauff[1] who tested a range of ingredients for their ability to stimulate growth of lactic acid bacteria. Tween® 80, liver extract, maltose, N-acetyl glucosamine and yeast extract were found to stimulate growth. Tomato juice, free fatty acids and lyophilized beer solids (all of which are found in several media formulations for lactic acid bacteria) were inhibitory.

In comparative studies using in-process beer samples, Raka-Ray media gave higher colony counts for lactobacilli than Tomato Juice Agar, W-L Differential Agar and Universal Beer Agar, with larger colonies developing after 2-4 days of anaerobic incubation[1,2]

Raka-Ray No. 3 Medium yields larger lactic acid bacterial colonies than Universal Beer Agar.[3] Raka-Ray No. 3 Medium also suppressed the growth of non-lactic acid, facultative bacteria such as *Aerobacter aerogenes* and *Flavobacterium proteus* that are often associated with lactic beer spoilage organisms.[3]

Raka-Ray No. 3 Medium is also recommended by the 'European Brewing Congress Analytical Microbiologica' for enumeration of lactobacilli and pediococci[4]. The broth and agar may be made more selective by the addition of 3 grams of 2-phenylethanol and 3 mg of cycloheximide (Actidione®) dissolved in a small quantity of acetone per liter of medium before autoclaving. Yeasts and gram-negative bacteria are suppressed, facilitating enumeration of the lactic bacterial flora.

User Quality Control

Identity Specifications

Raka-Ray No. 3 Broth

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 5.89% solution, soluble in distilled or deionized water with 1% Tween® 80. Solution is medium to dark amber, clear.

Reaction of 5.89% Solution at 25°C: pH 5.4 ± 0.2

Raka-Ray No. 3 Medium

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 7.49% solution, soluble in distilled or deionized water with 1% Tween® 80 upon boiling. Solution is medium to dark amber, clear to very slightly opalescent.

Reaction of 7.49% Solution at 25°C: pH 5.4 ± 0.2

Cultural Response

Prepare Raka-Ray No. 3 Broth or Medium with selective agents per label directions. Inoculate and incubate anaerobically at 27-30°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	1,000-2,000	none to poor
Lactobacillus brevis	367	30-300	good
Lactobacillus buchneri	11307	30-300	good
Pediococcus acidilactici	8042	30-300	good

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

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

Principles of Procedure

Polysorbate 80, Liver Digest, Maltose and other sugars, N-Acetyl Glucosamine and Yeast Extract stimulate the growth of lactobacilli. The optional addition of cycloheximide provides increased selectivity against yeasts and gam-negative bacteria.

Formula

Raka-Ray No. 3 Broth

Formula Per Liter

Ingredient	Amount
Bacto Yeast Extract	5 g
Bacto Tryptone	20 g
Liver Digest	1 g
Maltose Reagent	10 g
Fructose	5 g
Dextrose	5 g
Betaine Hydrochloride	2 g
Di-ammonium Citrate	2 g
L-Aspartic Acid	2.5 g
Magnesium Sulphate	0.98 g
Manganese Sulphate	0.42 g
Dipotassium Phosphate	2 g
N-Acetyl Glucosamine	0.5 g
Potassium Glutamate	2.5 g

Final pH 5.4 ± 0.2 at 25°C

Raka-Ray No. 3 Medium

Formula Per Liter

Ingredient	Amount
Bacto Yeast Extract	5 g
Bacto Tryptone	20 g
Liver Digest	1 g
Maltose Reagent	10 g
Fructose	5 g
Dextrose	5 g
Betaine Hydrochloride	2 g
Di-ammonium Citrate	2 g
Potassium Aspartate	2.5 g
Magnesium Sulphate	0.98 g
Manganese Sulphate	0.42 g
Dipotassium Phosphate	2 g
N-Acetyl Glucosamine	0.5 g
Potassium Glutamate	2.5 g
Bacto Agar	16 g

Final pH 5.4 ± 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

Raka-Ray No. 3 Broth
Raka-Ray No. 3 Medium

Materials Required but not Provided

Flasks with closures
Distilled or deionized water
Tween® 80
2-phenylethanol
Actidione®
Acetone
Autoclave
Waterbath (50°C)
Petri dishes
Sterile tubes
Anaerobic chamber

Method of Preparation

Raka-Ray No. 3 Broth

1. Suspend 58.9 grams in 1 liter of distilled or deionized water containing 10 ml Tween® 80. Dispense into tubes with closures.
2. Autoclave at 121°C for 15 minutes.

Raka-Ray No. 3 Medium

1. Suspend 74.9 grams in 1 liter of distilled or deionized water containing 10 ml Tween® 80.
2. Heat to boiling to dissolve.
3. To increase the selectivity of the medium, add 3 grams of 2-phenylethanol and 3 mg cycloheximide (Actidione®) per liter before autoclaving. Do not overheat.
4. Autoclave at 121°C for 15 minutes.
5. Pour 15-20 ml of Raka-Ray Medium into each Petri dish and allow to solidify.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Overlay Technique for Enumeration of Lactic Acid Bacteria

1. Inoculate 0.1 ml of the beer sample onto well-dried plates containing 15-20 ml Raka-Ray No. 3 Medium. Five replicates of each sample are recommended.
2. Spread over the surface of the medium using a sterile glass rod.
3. Overlay the surface with 4 ml of the molten sterilized medium cooled to 50°C.
4. Incubate plates at 27-30°C in an anaerobic (H_2/CO_2) atmosphere.

Results

Lactobacilli are visible after 48 hours incubation as smooth, moist colonies that are 1 mm in diameter. Incubate the medium for a total of 7 days to allow development of slow-growing *Pediococcus* strains.

If the number of colonies on each plate exceeds 300, the sample should be diluted 1:10 in sterile physiological saline and retested.

References

1. **Saha, R. B., R. J. Sondag, and J. E. Middlekauff.** 1974. An improved medium for the selective culturing of lactic acid bacteria. Proceedings of the American Society of Brewing Chemists. 9th Congress, 9-10.
2. **VanKeer, C., L. Van Melkebeke, W. Vertriest, G. Hoozee, and E. Van Schoonenberghe.** 1983. Growth of *Lactobacillus* species on different media. J. Inst. of Brewing **89**:360-363.
3. **Report of the Technical Subcommittee.** 1976. Microbiological Controls. J. Am. Soc. of Brewing Chemists **34**:93-94.
4. **European Brewing Congress Analytica Microbiologica.** 1981. J. Inst. of Brewing **87**:314.

Packaging

Raka-Ray No. 3 Broth	500 g	1865-17
Raka-Ray No. 3 Medium	500 g	1867-17

Rappaport-Vassiliadis Medium Semisolid

Bacto® Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification · Novobiocin Antimicrobic Supplement

Intended Use

Bacto Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification is is used with Bacto Novobiocin Antimicrobic Supplement in rapidly detecting motile *Salmonella* in feces and food products.

Summary and Explanation

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella* in feces and food products. The original work on

MSRV medium showed that a semi-solid medium in Petri dishes could be used as a rapid and sensitive means of isolating motile *Salmonella* from food products following pre-enrichment or selective enrichment.[1,2] The semisolid medium allows motility to be detected as halos of growth around the original point of inoculation.

The medium is recommended by the European Chocolate Manufacturer's Association. A collaborative study performed with support of the American Cocoa Research Institute (ACRI) and the Canadian Chocolate Manufacturer's Association (CCMA) resulted in first action adoption of the MSRV method by AOAC International.[3]

MSRV Medium may be used as a plating medium for isolating *Salmonella* spp. (other than *S. typhi* and *S. paratyphi* type A) from stool specimens with high sensitivity and specificity.[4,5]

Principles of the Procedure

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification contains Tryptose and Casein Hydrolysate as carbon and nitrogen sources for general growth requirements. Magnesium Chloride raises the osmotic pressure in the medium. Novobiocin (Novobiocin Antimicrobic Supplement) and Malachite Green inhibit organisms other than *Salmonella*. The low pH of the medium combined with the Novobiocin, Malachite Green and Magnesium Chloride select for highly resistant *Salmonella* spp. Bacto Agar is the solidifying agent.

Formula

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification

Formula per Liter

Bacto Tryptose	4.59	g
Casein Hydrolysate (Acid)	4.59	g
Sodium Chloride	7.34	g
Potassium Dihydrogen Phosphate	1.47	g
Magnesium Chloride Anhydrous	10.93	g
Malachite Green Oxalate	0.037	g
Bacto Agar	2.7	g

Final pH 5.2 ± 0.2 at 25°C

Novobiocin Antimicrobic Supplement

Formula per 10 ml

Sodium Novobiocin	20	mg

Precautions

1. For Laboratory Use.
2. **Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification**

   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): Nerves, Kidneys.

   Novobiocin Antimicrobic Supplement

   HARMFUL. HARMFUL BY INHALATION AND IF SWALLOWED. (EC) MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pale green, homogeneous, free-flowing.
Solution:	3.16% solution, soluble in distilled or deionized water upon boiling. Blue, clear to slightly opalescent.
Prepared Medium:	Blue, slightly opalescent, no significant precipitate, semisolid.
Reaction of 3.16% Solution at 25°C:	pH 5.2 ± 0.2

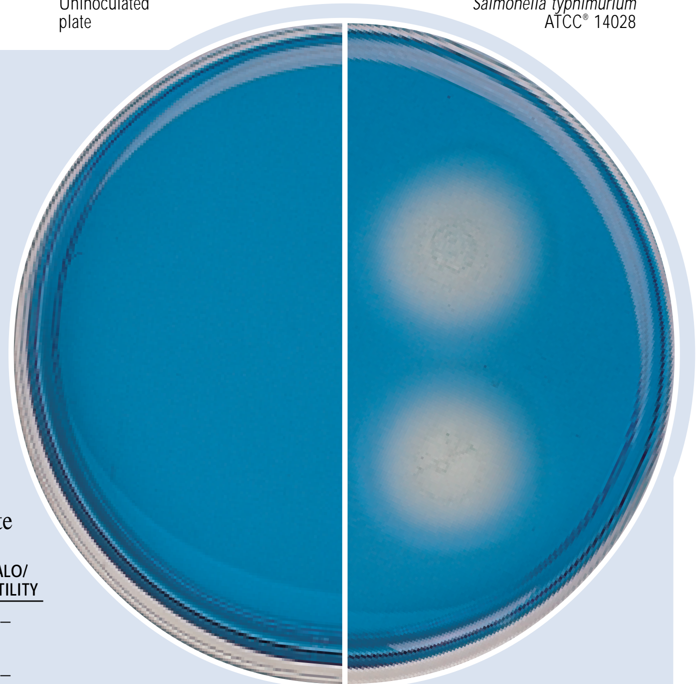
Cultural Response

Prepare Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification per label directions. Inoculate using three drops (approximately 0.1 ml) at discreet locations on the plate and incubate at 42 ± 0.5°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HALO/ MOTILITY
Citrobacter freundii	8090	1,000-2,000	marked to complete inhibition	–
Pseudomonas aeruginosa	27853*	1,000-2,000	none	–
Salmonella typhimurium	14028*	100-1,000	good	+
Salmonella senftenberg (NCTC)	10384	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 plate

Salmonella typhimurium ATCC® 14028

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 Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store Novobiocin Antimicrobic Supplement at 2-8°C.

Expiration Date

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

Procedure

Materials Provided

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification

Novobiocin Antimicrobic Supplement

Materials Required but not Provided

Flask with closure

Distilled or deionized water

Autoclave

Incubator (35°C)

Waterbath

Method of Preparation

1. Suspend 31.6 grams of Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Do not autoclave.
3. Cool to 50°C.
4. Aseptically add 10 ml Novobiocin Antimicrobic Supplement, rehydrated per label instructions with sterile distilled or deionized water. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[3,6]

Pre-enrichment

1. Add 25 grams of cocoa or chocolate to 225 ml of sterile reconstituted nonfat dry milk with 0.45 ml of a 1% aqueous brilliant green dye solution; mix well.[6]
2. Incubate at 35°C for 20 ± 2 hours.[3]

Selective Enrichment[3]

3. Inoculate 10 ml Tetrathionate Broth (prewarmed to 35°C) with 1 ml of the pre-enrichment culture.
4. Incubate at 35°C for 8 ± 0.5 hours.

Motility Enrichment on MSRV[3]

5. After selective enrichment incubation, mix the broth culture. Inoculate 3 drops at separate spots on an MSRV plate.
6. Incubate at 42 ± 0.5°C for 16 ± 0.5 hours.

Results

Positive: Growth of migrated cells is visible as a gray-white, turbid zone extending out from the inoculated drop. Test sample is considered presumptively positive for motile *Salmonella*.

Negative: Medium remains blue-green around the drops, with no gray-white, turbid zone extending out from the drop. Test sample is considered negative for motile *Salmonella*.

To confirm a presumptive identification of *Salmonella*:[3]

Rapid serologic confirmation

1. Inoculate M Broth with growth from migration edge on MSRV plate.
2. Incubate at 35°C for 4 to 6 hours (until turbid). M-broth culture can be held for up to 24 hours at 35°C.
3. Test with *Salmonella* O and H antisera.

Culture confirmation

1. Transfer a loopful of growth from the migration edge on MSRV plate onto Hektoen Enteric Agar and streak for isolation.
2. Incubate at 35°C for 24 ± 2 hours.
3. From colonies of Hektoen agar that show colony appearance typical of *Salmonella* (green colonies with black centers), perform biochemical tests to confirm the identification.

Limitations of the Procedure

The combination of malachite green, magnesium chloride and a low pH may inhibit certain *Salmonella*, such as *S. typhi* and *S. choleraesuis*. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. **DeSmedt, J. M., R. Bolderdijk, H. Rappold, and D. Lautenschlaeger.** 1986. Rapid *Salmonella* detection in foods by motility enrichment on a modified semi-solid Rappaport-Vassiliadis medium. J. Food Prot. **49**:510-514.
2. **DeSmedt, J. M., and R. Bolderdijk**. 1987. Dynamics of *Salmonella* isolation with modified semi-solid Rappaport-Vassiliadis medium. J. Food Prot. **50**:658- 661.
3. **DeSmedt, J. M., R. Bolderdijk, and J. Milas**. 1994. *Salmonella* detection in cocoa and chocolate by motility enrichment on modified semi-solid Rappaport-Vassiliadis medium: a collaborative study. J. AOAC Int. **77**:365-373.
4. **Dusch, H., and M. Altwegg**. 1995. Evaluation of five new plating media for isolation of *Salmonella* sp. J. Clin. Micro. **33**:802-804.
5. **Aspinall, S. T., M. A. Hindle, and D. N. Hutchinson**. 1992. Improved isolation of Salmonellae from faeces using a semi-solid Rappaport-Vassiliadis Medium. Eur. J. Clin. Microbiol. Infect. Dis. **11**:936-939.
6. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella.* p. 5.01-5.20. *In* FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification	500 g	1868-17
Novobiocin Antimicrobic Supplement	6 x 10 ml	3197-60*

*Store at 2-8%C

Bacto® Rappaport-Vassiliadis R10 Broth

Intended Use

Bacto Rappaport-Vassiliadis R10 Broth is used for selectively enriching *Salmonella* from meat and dairy products, feces and sewage polluted water.

Also Known As

Rappaport-Vassiliadis R10 Broth is also known as RV Enrichment Broth or R10 Broth.

Summary and Explanation

Rappaport et al[1] formulated an enrichment medium for *Salmonella* that was modified by Vassiliadis et al.[2] The Rappaport formulation, designated R25/37°C, recommended incubation at 37°C; the Vassiliadis modification, designated R10/43°C, had a reduced level of malachite green and recommended incubation at 43°C. Later work by Peterz showed that incubation at 41.5° ± 0.5°C for 24 hours improved recovery of *Salmonella* spp.[3]

Rappaport-Vassiliadis R10 Broth is a selective enrichment medium that is used following pre-enrichment of the specimen in a suitable pre-enrichment medium. It has gained approval for use in analyzing milk and milk products,[4] raw flesh foods, highly contaminated foods and animal feeds.[5,6]

This medium selectively enriches for salmonellae because bacteria, including other intestinal bacteria, are typically resistant to or inhibited by malachite green, high osmotic pressure and/or low pH. *S. typhi* and *S. choleraesuis* are sensitive to malachite green and may be inhibited.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pale green to green, free-flowing, homogeneous.
Solution:	2.66% solution, soluble in distilled or deionized water upon gentle heating; blue, clear.
Reaction of 2.66% Solution at 25°C:	pH 5.1 ± 0.2

Cultural Response

Prepare Rappaport-Vassiliadis R10 Broth per label directions. Inoculate and incubate at 41.5 ± 0.5°C for 18-48 hours. Subculture to Brilliant Green Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Escherichia coli	25922*	1,000-2,000	markedly inhibited
Salmonella enteritidis	13076	100-1,000	good
Salmonella typhimurium	14028*	100-1,000	good

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

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

Principles of the Procedure

Rappaport-Vassiliadis R10 Broth contains Tryptone as carbon and nitrogen sources for general growth requirements. Magnesium Chloride raises the osmotic pressure in the medium. Malachite Green is inhibitory to organisms other than salmonellae. The low pH of the medium (5.1 ± 0.2 at 25°C), combined with the presence of malachite green and magnesium chloride, select for the highly resistant *Salmonella* spp.

Formula

Rappaport-Vassiliadis R10 Broth

Formula Per Liter

Bacto Tryptone	4.54	g
Sodium Chloride	7.2	g
Potassium Dihydrogen Phosphate	1.45	g
Magnesium Chloride Anhydrous	13.4	g
Malachite Green Oxalate	0.036	g

Final pH 5.1 ± 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): Nerves, Kidneys.

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Rappaport-Vassiliadis R10 Broth

Materials Required but not Provided

Flask with closure
Containers suitable for 10 ml aliquots
Distilled or deionized water
Autoclave

Method of Preparation

1. Suspend 26.6 grams in 1 liter distilled or deionized water. Heat gently to dissolve.
2. Dispense 10 ml amounts into suitable containers. Sterilize at 115-116°C for 15 minutes.

Specimen Preparation

Consult an appropriate reference for specific instructions related to the type of product being tested.[4,5,6]

Test Procedure

Water and Sewage Samples

For isolating *Salmonella* (other than *S. typhi*) from water and associated materials such as sewage liquor, sewage sludge, digested sludge and pressed sludge cake.

1. Concentrate the sample by filtering it through a plug of sterile absorbent cottonwool inserted in the neck of a large sterile funnel or through a Whatman No. 17 absorbent pad.

Pre-enrichment

2. Using aseptic technique, transfer the cottonwool plug or the pad to 100 ml of a suitable pre-enrichment medium such as Buffered Peptone Water.
3. Incubate at 37 ± 0.5°C for 18-24 hours.

Selective Enrichment

4. Inoculate 10 ml of Rappaport-Vassiliadis R10 Broth with 0.1 ml of the pre-enrichment culture. Inoculate 10 ml of Muller-Kauffman Tetrathionate Broth with 1 ml of the pre-enrichment culture.
5. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C. Incubate Muller- Kauffman Tetrathionate Broth at 42 ± 1°C for 48 hours.

Results

6. After incubation, subculture both selective enrichment broths to Brilliant Green Agar and XLD Agar. Incubate at 35 ± 2°C for 18-24 hours.
7. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

Milk and Foods

For isolating *Salmonella* (other than *S. typhi*) from milk and milk products,[4] raw flesh foods, highly contaminated foods and animal feeds.[5,6]

Pre-enrichment

1. Add 25 grams or a 25 ml sample of the specimen to 225 ml of pre-enrichment medium. Consult appropriate references for the type of product being tested.[4,5,6]
2. Incubate at 35°C for 24 ± 2 hours[5,6] or at 37°C for 16-20 hours,[4] depending on the referenced procedure being followed.

Selective Enrichment

1. Inoculate 10 ml of Rappaport-Vassiliadis R10 Broth with 0.1 ml of pre-enrichment culture. Inoculate 10 ml of another selective enrichment medium such as Tetrathionate Broth or Selenite Cystine Broth with 1 ml of the pre-enrichment culture.[4,5,6]
2. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C[4] for 24 ± 2 hours. Incubate the other selective enrichment broths appropriately.

Results

1. After incubation, subculture Rappaport-Vassiliadis R10 Broth and the other selective enrichment broths to selective agar media and incubate at 35 ± 2°C for 24 ± 2 hours.[4,5]
2. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

Limitations of the Procedure

The combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain *Salmonella*, such as *S. typhi* and *S. choleraesuis*. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. **Rappaport, F., N. Konforti, and B. Navon**. 1956. A new enrichment medium for certain salmonellae. J. Clin. Pathol. **9**:261-266.
2. **Vassiliadis, P., D. Trichopoulos, A. Kalandidi, and E. Xirouchaki**. 1978. Isolation of salmonellae from sewage with a new procedure of enrichment. J. Appl. Bacteriol. **44**:233-239.
3. **Peterz, M., C. Wiberg, and P. Norberg**. 1989. The effect of incubation temperature and magnesium chloride concentration on growth of salmonella in home-made and commercially available dehydrated Rappaport-Vassiliadis broths. J. Appl. Bacteriol. **66**:523-528.
4. **International Dairy Federation**. 1995. Milk and milk products: detection of *Salmonella*. IDF Standard **93B**:1005. Brussels, Belgium.
5. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella.* p. 5.01-5.20. *In* FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Andrews, W. H.** (ed.). 1995. Microbial methods, p.1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Rappaport-Vassiliadis R10 Broth	500 g	1858-17

Bacto® Reinforced Clostridial Medium

Intended Use

Bacto Reinforced Clostridial Medium is used for cultivating and enumerating clostridia, other anaerobes, and other species of bacteria from foods and clinical specimens.

Summary and Explanation

Reinforced Clostridial Medium is a semisolid medium formulated by Hirsch and Grinstead.[1] Their work demonstrated that the medium outperformed other media in supporting growth of clostridia from small inocula and produced higher viable cell counts.[1] Barnes and Ingram[2]

used the medium to dilute vegetative cells of *Clostridium perfringens*. Barnes et al[3] used a solid (agar) version of the medium to enumerate clostridia in food. The medium is a non-selective enrichment medium and grows various anaerobic and facultative bacteria when incubated anaerobically.[4]

Principles of the Procedure

Reinforced Clostridial Agar contains Tryptose and Beef Extract as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Sodium Chloride maintains the osmotic balance. In low concentrations, Soluble Starch detoxifies metabolic by-products. Cysteine Hydrochloride is the reducing agent. Sodium Acetate acts as a buffer. The small amount of Bacto Agar makes the medium semisolid.

Formula

Reinforced Clostridial Medium

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	10 g
Bacto Yeast Extract	3 g
Bacto Dextrose	5 g
Sodium Chloride	5 g
Soluble Starch	1 g
Cysteine Hydrochloride	0.5 g
Sodium Acetate	3 g
Bacto Agar	0.5 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

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

Solution: 3.8% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, slightly opalescent. Upon cooling medium becomes more opalescent.

Reaction of 3.8% Solutionat 25°C: pH 6.8 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis	23745	100-1,000	good
Clostridium botulinum	25763	100-1,000	good
Clostridium perfringens	13124*	100-1,000	good

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

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

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

Reinforced Clostridial Medium

Materials Required but not Provided

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

Method of Preparation

1. Suspend 38 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

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. **Hirsch, A., and E. Grinstead**. 1954. Methods for the growth and enumeration of anaerobic spore formers from cheese, with observations on the effect of nisin. J. Dairy Res. **21**:101-110.
2. **Barnes, E. M., and M. Ingram**. 1956. The effect of redox potential on the grown *Clostridium welchii* strain isolated from horse muscle. J. Appl. Bacteriol. **19**:117- 128.
3. **Barnes, E. M., J. E. Despaul, and M. Ingram**. 1963. The behavior of a food poisoning strain of *Clostridium welchii* in beef. J. Appl. Bacteriol. **26**:415.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 660-668. Williams & Wilkins, Baltimore, MD.

Packaging

Reinforced Clostridial Medium	500 g	1808-17

Bacto® Riboflavin Assay Medium

Intended Use

Riboflavin Assay Medium is used for determining riboflavin 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 maintaining the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To permit quantitation of the vitamin under test.

Riboflavin Assay Medium is a modification of the medium described by Snell and Strong.[1] It is recommended for use in the microbiological assay of riboflavin following the methodology outlined by the U.S. Food and Drug Administration[2] using *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469 as the test organism.

Principles of the Procedure

Riboflavin Assay Medium is free from riboflavin but contains all other nutrients and vitamins essential for the growth of *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469. The addition of riboflavin in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Riboflavin Assay Medium

Formula Per Liter

Ingredient	Amount	Unit
Bacto Dextrose	20	g
Sodium Acetate	15	g
Bacto Vitamin Assay Casamino Acids	10	g
Dipotassium Phosphate	1	g
Monopotassium Phosphate	1	g
L-Asparagine	0.6	g
DL-Tryptophane	0.2	g
L-Cystine	0.2	g
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Xanthine	20	mg
Magnesium Sulfate USP	0.4	g
Ferrous Sulfate	20	mg
Manganese Sulfate Monohydrate	20	mg
Sodium Chloride USP	20	mg
Pyridoxine Hydrochloride	4	mg
Pyridoxal Hydrochloride	4	mg
p-Aminobenzoic Acid	2	mg
Calcium Pantothenate	800	µg
Folic Acid	800	µg
Nicotinic Acid	800	µg
Thiamine Hydrochloride	400	µg
Biotin	1	µg

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 2.4% solution (single strength) and 4.8% (double strength), soluble in distilled or deionized water on boiling. Light to medium amber, clear, may have a slight precipitate.

Prepared Medium: Light amber, clear, may have a very slight precipitate.

Reaction of 2.4% Solution at 25°C: pH 6.8 ± 0.2

Cultural Response

Prepare Riboflavin Assay Medium per label directions. The medium supports the growth of *L. casei* subsp. *rhamnosus* ATCC® 7469 when prepared in single strength and supplemented with riboflavin. Measure growth response turbidimetrically with increasing concentrations of riboflavin to produce a standard curve.

Precautions

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

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

Riboflavin Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469
Sterile tubes
Sterile 0.85% saline
Distilled or deionized water
Lactobacilli Agar AOAC or Micro Assay Culture Agar
Lactobacilli Broth AOAC or Micro Inoculum Broth
Riboflavin USP
Spectrophotometer

Method of Preparation

1. Suspend 4.8 grams in 100 ml of distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

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

Test Procedure

Follow assay procedures as outlined in AOAC.[1] Levels of riboflavin used in the determination of the standard curve should be prepared according to this reference or according to the following procedure.

Stock Cultures

Stock cultures of *L. casei* subsp. *rhamnosus* ATCC® 7469 are prepared by stab inoculation into 10 ml of Lactobacilli Agar AOAC. After 24-48 hours incubation at 35-37°C, the stock cultures are kept in the refrigerator. Transfers are made at monthly intervals in triplicate.

Inoculum

Inoculum for assay is prepared by subculturing a stock culture of *L. casei* subsp. *rhamnosus* ATCC® 7469 into 10 ml of Lactobacilli Broth AOAC or Micro Inoculum Broth. Following incubation for 16-24 hours at 35-37°C, the culture is centrifuged under aseptic conditions and the supernatant liquid decanted. After washing 3 times with 10 ml sterile 0.85% saline, the cells are resuspended in 10 ml sterile 0.85% saline. The cell suspension is then diluted with sterile 0.85% saline, to a turbidity of 35-40% transmittance when read on the spectrophotometer at 660 nm. One drop of this latter suspension is then used to inoculate each of the assay tubes.

Riboflavin Assay Medium may be used for both turbidimetric and titrimetric determinations. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C, where as titrimetric determinations are best made after 72 hours incubation at 35-37°C. Using Riboflavin Assay Medium, the most effective assay range is between 0.025 and 0.15 µg riboflavin.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation, which influence the standard curve readings, cannot be duplicated exactly from assay to assay. The standard curve is obtained by using Riboflavin USP Reference Standard or equivalent at levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 and 0.3 µg riboflavin per assay tube (10 ml).

The concentration of riboflavin required for the preparation of the standard curve may be prepared by dissolving 0.1 g of Riboflavin USP Reference Standard or equivalent in 1,000 ml of distilled water by heating, giving a stock solution of 100 µg per ml. Dilute the stock solution by adding 1 ml to 999 ml distilled water. Use 0.0, 0.25, 0.5, 0.75, 1, 1.5, 2 and 3 ml of the diluted stock solution per tube. Prepare the stock solution fresh daily.

Results

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

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.
5. Maintain pH below 7.0 to prevent loss of riboflavin.

References

1. **Snell and Strong.** 1939. Ind. and Eng. Chem. **11**:346.
2. **Association of Analytical Chemists.** 1996. U.S. Food and Drug Administration methods or the microbiological analysis of selected nutrients. AOAC Internationl, Gaithersburg, MD.

Packaging

Riboflavin Assay Medium	100 g	0325-15*

*Store at 2-8°C

Bacto® Rice Extract Agar

Intended Use

Bacto Rice Extract Agar is used for differentiating *Candida albicans* and other *Candida* spp. based on chlamydospore formation.

Summary and Explanation

Rice Extract Agar is prepared according to the formulation of Taschdjian.[1] Chlamydospores were observed consistently and in abundance upon this medium 17-24 hours after inoculation with *Candida albicans*. The morphology of both the pathogenic and nonpathogenic *Candida* agreed in every respect with that of the cultures grown on corn meal agar; corn meal agar was the medium most routinely used in laboratories at that time, but was time-consuming and laborious to prepare. In later studies Taschdjian[2] and Kelly and Funigiello[3] showed that the addition of Tween® 80 (polysorbate 80) to Rice Extract Agar enhanced chlamydospore formation by *C. albicans*. The addition of 2% dextrose enhanced pigment production by *Trichophyton rubrum* permitting the differentiation between this dermatophyte and *Trichophyton mentagrophytes*.

Taubert and Smith[4] recommended Rice Extract Agar for use in the diagnosis of vulvovaginal candidiasis. A cotton-tipped applicator was

used for obtaining a specimen and then rolled on the surface of a rice extract agar plate; a cover glass was then applied to the agar, covering most of the inoculum.

Principles of the Procedure

The Rice Extract provides the sole source of nutrients in the medium. This lack of nutrients together with the oxygen-deficient culture conditions (covering the inoculum with a cover glass) creates a deficient environment that induces the formation of specific morphological forms (chlamydospores and pseudomycelia in particular) in some yeasts. The addition of Tween 80 further stimulates chlamydospore formation due to its content of oleic acids. Bacto Agar is incorporated into the medium as a solidifying agent.

Formula

Rice Extract Agar

Formula Per Liter

White Rice, Extract from	20 g
Bacto Agar	20 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.5% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, opalescent with precipitation.
Prepared Medium:	Colorless to light amber, opaque, precipitate.
Reaction of 2.5% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare Rice Extract Agar per label directions. Inoculate and incubate at 23-25°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	CHLAMYDOSPORES
Candida albicans	10231	30-300	good	+
Candida albicans	26790	30-300	good	+

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

Procedure

Materials Provided

Rice Extract Agar

Materials Required But Not Provided

Glassware
Autoclave
Distilled or deionized water

Method of Preparation

1. Suspend 25 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 dispense medium into sterile Petri dishes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate the plates by cutting through the surface of the agar with an inoculating wire.
2. Cover the inoculated area with a sterile cover slip.
3. Invert plates and incubate at 23-25°C for 18-72 hours.
4. Examine for chlamydospores microscopically using approximately 100X magnification and by focusing upon the line of inoculation.

Results

After 24 to 48 hours most strains of *C. albicans* and *C. stellatoidea* will have formed typical chlamydospores.[5]

Limitations of the Procedure

1. Further studies should be performed to confirm the results obtained.
2. Tween 80 enhances chlamydospore production in many species of *Candida.* It is therefore necessary to use additional media for species identification.[6]
3. High temperatures for incubation should be avoided as chlamydospores are not formed at 37°C.

References

1. **Taschdjian, C. L.** 1953. A simple prepared identification medium for *Candida albicans.* Mycologia **45**:474.
2. **Taschdjian, C. L.** 1957. Routine identification of *Candida albicans*: Current methods and a new medium. Mycologia **49**:332.
3. **Kelly, J. P., and F. Funigiello.** 1959. *Candida albicans*: A study of media designed to promote chlamydospore production. J. Lab. Clin. Med. **53**:807-809.
4. **Taubert, H. D., and A. G. Smith.** 1960. The clinical use of Taschdjian's medium in the diagnosis of vulvovaginal candidiasis. J. Lab. Clin. Med. **55**:820- 828.
5. **Cooper, and Silva-Hutner**. 1985. *In* Lennette, Balows, Hausler, and Shadomy (ed.), Manual of clinical microbiology, 4th ed. ASM, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Rice Extract Agar	500 g	0899-17

Bacto® Rogosa SL Agar
Bacto Rogosa SL Broth

Intended Use

Bacto Rogosa SL Agar and Bacto Rogosa SL Broth are used for cultivating oral, vaginal and fecal lactobacilli.

Also Known As

Rogosa SL Agar is also known as RMW Agar.

Summary and Explanation

Rogosa SL Agar and Broth are a modification of media described by Rogosa, Mitchell and Wiseman.[1,2] These media are used for isolation, enumeration and identification of lactobacilli in oral bacteriology, feces, vaginal specimens and foodstuffs.[3,4] The low pH and high acetate concentrations effectively suppress other bacterial flora allowing lactobacilli to flourish.

User Quality Control

Identity Specifications

Rogosa SL Agar

Dehydrated Appearance: Beige, homogeneous with soft clumps.

Solution: 7.5% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, slightly opalescent and may have a slight precipitate.

Reaction of 7.5% Solution at 25°C: pH 5.4 ± 0.2

Rogosa SL Broth

Dehydrated Appearance: Beige, appears moist, slightly lumpy.

Solution: 6.0% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, clear to slightly opalescent.

Reaction of 6.0% Solution at 25°C: pH 5.4 ± 0.2

Cultural Response

Rogosa SL Agar

Prepare Rogosa SL Agar per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

Rogosa SL Broth

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus casei	9595	100-1,000	good
Lactobacillus delbrueckii	4797	100-1,000	good
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition

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 provides carbon and nitrogen. Yeast Extract is a source of trace elements, vitamins and amino acids. Dextrose, Arabinose and Saccharose are carbohydrate sources that provide carbon. Sodium Acetate and Ammonium Citrate inhibit streptococci, molds and other oral microbial flora and restrict swarming. Monopotassium Phosphate provides buffering capability. Magnesium Sulfate, Manganese Sulfate and Ferrous Sulfate are sources of inorganic ions. Sorbitan Monooleate (Polysorbate 80) acts as a surfactant. Bacto Agar is a solidifying agent.

Formula

Rogosa SL Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Bacto Arabinose	5 g
Bacto Saccharose	5 g
Sodium Acetate	15 g
Ammonium Citrate	2 g
Monopotassium Phosphate	6 g
Magnesium Sulfate	0.57 g
Manganese Sulfate	0.12 g
Ferrous Sulfate	0.03 g
Sorbitan Monooleate	1 g
Bacto Agar	15 g

Final pH 5.4 ± 0.2 at 25°C

Rogosa SL Broth

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Bacto Arabinose	5 g
Bacto Saccharose	5 g
Sodium Acetate	15 g
Ammonium Citrate	2 g
Monopotassium Phosphate	6 g
Magnesium Sulfate	0.57 g
Manganese Sulfate	0.12 g
Ferrous Sulfate	0.03 g
Sorbitan Monooleate	1 g

Final pH 5.4 ± 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 dehydrated media at 2-8°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

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

Procedure

Materials Provided

Rogosa SL Agar

Rogosa SL Broth

Materials Required but not Provided

Glassware

Distilled or deionized water

Glacial acetic acid

Incubator (35°C)

Method of Preparation

Rogosa SL Agar

1. Suspend 75 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 1.32 ml glacial acetic acid and mix well.
4. Boil 2-3 minutes. DO NOT AUTOCLAVE.

Rogosa SL Broth

1. Suspend 60 grams in 1 liter distilled or deionized water.
2. Add 1.32 ml glacial acetic acid and mix well.
3. Boil 2-3 minutes. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

The salt in the formulation makes the media not suitable for isolation of dairy lactobacilli; e.g., *L. lactis, L. bulgaricus and L. helveticus*.[4]

References

1. **Rogosa, M., J. A. Mitchell, and R. F. Wiseman.** 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. **62**:132.
2. **Rogosa, M., J. A. Mitchell, and R. F. Wiseman.** 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Dental Res. **30**:682.
3. **Vedamuthu, E. R., M. Raccach, B. A. Glatz, E. W. Seitz, and M. S. Reddy.** 1992. Acid-producing Microorganisms. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Assoc., Washington, D.C.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 678-680. Williams & Wilkins, Baltimore, MD.

Packaging

Rogosa SL Agar	500 g	0480-17
	10 kg	0480-08
Rogosa SL Broth	500 g	0478-17

Rose Bengal Agar

Bacto® Rose Bengal Agar Base · Bacto Rose Bengal Antimicrobic Supplement C

Intended Use

Bacto Rose Bengal Agar Base is used with Bacto Rose Bengal Antimicrobic Supplement C in isolating and enumerating yeasts and molds.

Also Known As

Rose Bengal Agar is also known as Rose Bengal Chloramphenicol Agar and Rose Bengal-Malt Extract Agar.

Summary and Explanation

A number of methods have been described for the selective isolation of fungi from environmental materials and foodstuffs containing mixed populations of fungi and bacteria. The use of media with an acid pH that selectively inhibits the growth of bacteria and thereby promotes the growth of fungi has been widely employed.[1,2,3] A number of investigators have reported, however, that acidified media may actually inhibit fungal growth,[4,5] fail to completely inhibit bacterial growth,[5] and have little effect in restricting the size of mold colonies.[6] Smith and Dawson[7] used Rose Bengal in a neutral pH medium for the selective isolation of fungi from soil samples. Chloramphenicol, streptomycin, oxytetracycline and chlortetracycline have been used for the improved, selective isolation and enumeration of yeasts and molds from soil, sewage and foodstuffs.[4,8,9,10,11]

Rose Bengal Agar Base supplemented with Rose Bengal Antimicrobic Supplement C is a modification of the Rose Bengal Chlortetracycline Agar formula of Jarvis.[11] Instead of chlortetracycline, chloramphenicol is employed in this medium as a selective supplement. Of the antibiotics most frequently employed in media of neutral pH, chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum.[12] Rose Bengal Agar is recommended in standard methods for the enumeration of yeasts and molds from foodstuffs and water.[12,13,14,15]

Principles of the Procedure

Soytone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms. Dextrose is an energy source.

Monopotassium Phosphate provides buffering capability. Magnesium Sulfate provides necessary trace elements. Rose Bengal is included as a selective agent that inhibits bacterial growth and restricts the size and height of colonies of the more rapidly growing molds. The restriction in growth of molds aids in the isolation of slow-growing fungi by preventing overgrowth by more rapidly growing species. Rose Bengal is taken up by yeast and mold colonies, thereby facilitating their recognition and enumeration. Rose Bengal Antimicrobic Supplement C is a lyophilized antimicrobic supplement containing chloramphenicol which inhibits bacteria. Bacto Agar is the solidifying agent.

Formula

Rose Bengal Agar Base

Formula Per Liter

Bacto Soytone	5 g
Bacto Dextrose	10 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Rose Bengal	0.05 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Rose Bengal Antimicrobic Supplement C

Formula Per 2 ml Vial

Chloramphenicol	0.05 g

Precautions

1. For Laboratory Use.
2. **Rose Bengal Antimicrobic Supplement C**

   TOXIC. MAY CAUSE CANCER. MAY CAUSE HERITABLE GENETIC DAMAGE. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. MAY CAUSE SENSITIZATION BY INHALATION AND SKIN CONTACT. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately. (Show label where possible.) Do not breathe dust. Keep container tightly closed. Target Organs: Blood, Bone Marrow.

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

Storage

Store **Rose Bengal Agar Base** dehydrated below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

Store **Rose Bengal Antimicrobic Supplement C** at 2-8°C. Do not open or rehydrate vials until ready to use. Store rehydrated vials at 2-8°C and use within 24 hours.

User Quality Control

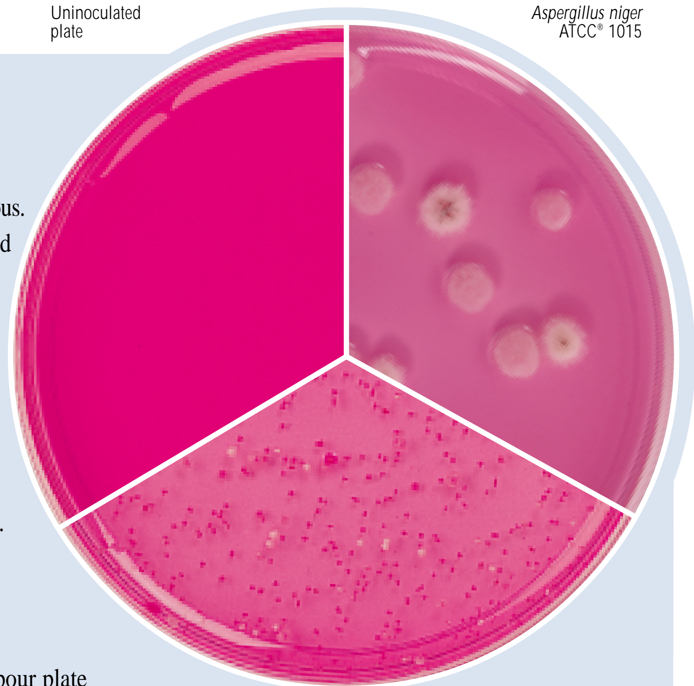
Identity Specifications

Rose Bengal Agar Base

Dehydrated Appearance:	Beige to faint pink, free-flowing, homogeneous.
Solution:	3.2% solution, soluble in distilled or deionized water on boiling. Solution is reddish pink, very slightly to slightly opalescent.
Complete Prepared Medium:	Bright pink, very slightly to slightly opalescent.
Reaction of 3.2% Solution at 25°C:	pH 7.2 ± 0.2

Rose Bengal Antimicrobic Supplement C

Lyophilized Appearance:	Lyophilized white cake, may be dispersed.
Rehydrated Appearance:	Colorless, clear.
Solubility:	Soluble in 2 ml ethanol.



Cultural Response

Prepare Rose Bengal Agar per label directions. Inoculate using the pour plate technique (for *Aspergillus niger*, inoculate the surface of an agar slant) and incubate aerobically at 25-30°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	COLONY COLOR
Aspergillus niger	1015	100-300	good	white
Candida albicans	10231	100-300	good	pink
Escherichia coli	25922*	1,000-2,000	inhibited	–
Micrococcus luteus	10240	1,000-2,000	inhibited	–

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

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

Expiration Date

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

Procedure

Materials Provided

Rose Bengal Agar Base
Rose Bengal Antimicrobic Supplement C

Materials Required But Not Provided

Glassware
Autoclave
Incubator (25°C)
Sterile Petri dishes
Ethanol (reagent grade)
Bent glass rods

Method of Preparation

1. **Rose Bengal Antimicrobic Supplement C:** To rehydrate, aseptically add 2 ml of ethanol per vial of dehydrated supplement and invert several times to dissolve the powder.
2. **Rose Bengal Agar Base:** To rehydrate, suspend 16 grams in 500 ml distilled or deionized water.
3. Heat to boiling to dissolve completely.
4. Sterilize the basal medium at 121°C for 15 minutes and then cool to 45-50°C.
5. Aseptically add 2 ml of the rehydrated Rose Bengal Antimicrobic Supplement C to 500 ml of cooled agar base. Mix thoroughly.
6. Dispense into sterile Petri dishes and allow to dry overnight at room temperature (21-25°C).

Specimen Collection and Preparation

Collect specimens in sterile containers and transport immediately to the laboratory in accordance with recommended guidelines.[12,13] Prepare samples for dilution plating inoculation. It is recommended that yeast and molds be enumerated by a surface spread-plate technique rather than with pour plates.[12] The spread-plate technique provides maximal exposure of cells to atmospheric oxygen and eliminates heat stress from molten agar.[12]

Test Procedure

1. Inoculate 0.1 ml of appropriate dilutions in duplicate on the solidified agar. Spread over the entire surface using a sterile bent glass rod.
2. Incubate plates at 25-30°C for up to 7 days.

Results

Colonies of yeast appear pink due to the uptake of rose bengal. Count plates containing 15 to 150 colonies and report the counts as colony forming units (CFU) per gram or ml of sample.

Limitations of the Procedure

1. Although this medium is selective primarily for fungi, microscopic examination is recommended for presumptive identification. Biochemical testing using pure cultures is required for complete identification.
2. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the complete medium; similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
3. Care should be taken not to expose this medium to light since photodegradation of rose bengal yields compounds that are toxic to fungi.

References

1. **Waksman, S. A.** 1922. A method for counting the number of fungi in the soil. J. Bacteriol. **7**:339-341.
2. **Koburger, J. A.** 1976. Yeasts and molds, p. 225-229. *In* M. L. Speck (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
3. **Mossel, D. A. A., M. Visser, and W. H. J. Mengerink.** 1962. A comparison of media for the enumeration of moulds and yeasts in foods and beverages. Lab Practice **11**:109-112.
4. **Martin, J. P.** 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. **69**:215-232.
5. **Koburger, J. A.** 1972. Fungi in foods. IV. Effect of plating medium pH on counts. J. Milk Food Technol. **35**:659-660.
6. **Tyner, L. E.** 1944. Effect of media compositions on the numbers of bacterial and fungal colonies developing in Petri plates. Soil Sci. **57**:271-274.
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8. **Cooke, W. B.** 1954. The use of antibiotics in media for the isolation of fungi from polluted water. Antibiotics and Chemotherapy **4**:657-662.
9. **Papavizas, G. C., and C. B. Davey.** 1959. Evaluation of various media and antimicrobial agents for isolation of soil fungi. Soil Sci. **88**:112-117.
10. **Overcast, W. W., and D. J. Weakley.** 1969. An aureomycin-rose bengal agar for enumeration of yeast and mold in cottage cheese. J. Milk Technol. **32**:442-445.
11. **Jarvis, B.** 1973. Comparison of an improved rose bengal-chlortetracycline agar with other media for the selective isolation and enumeration of molds and yeasts in foods. J. Appl. Bact. **36**:723-727.
12. **Mislivec, P. B., L. R. Beuchat, and M. A. Cousin.** 1992. Yeasts and Molds. *In* C. Vanderzant and D. F. Splittstoesser (eds.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Assoc., Washington, D.C.
13. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Assoc., Washington, D.C.
14. **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.
15. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Rose Bengal Agar Base	500 g	1831-17
	10 kg	1831-08
Rose Bengal Antimicrobic Supplement C	6 x 2 ml	3352-54

Bacto® SABHI Agar Base

Intended Use

Bacto SABHI Agar Base is for use with chloromycetin and blood (optional) in isolating and cultivating pathogenic fungi.

Summary and Explanation

Sabouraud[1] formulated Sabouraud Dextrose Agar as a general purpose medium for the recovery of dermatophytes. Brain Heart Infusion is a highly nutritive medium used for cultivating a variety of fastidious organisms and medically important fungi.[2] SABHI Agar Base, prepared according to the formulation of Gorman[3], combines the ingredients from both Sabouraud Dextose Agar and Brain Heart Infusion. It is particularly useful for maximum recovery of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from body tissues and fluids and as a primary recovery medium for saprophytic and pathogenic fungi.[4]

Gorman reported that the addition of blood to this medium increased recovery and conversion to the yeast phase of *H. capsulatum* and *B. dermatitidis*.[3,5] Selectivity can be obtained by adding chloromycetin or other antimicrobics to the medium.[5]

Principles of the Procedure

Infusions from Calf Brains and Beef Heart are sources of carbon, protein and nutrients. Proteose Peptone and Neopeptone are sources of nitrogen, amino acids and carbon. Dextrose is an additional carbon source. Sodium Chloride provides essential ions while maintaining osmotic balance. Disodium Phosphate provides buffering capacity. Bacto Agar is a solidifying agent. Chloromycetin, when added, is a broad spectrum antibiotic that inhibits a wide variety of gram-negative bacteria.

Formula

SABHI Agar Base

Formula Per Liter

Calf Brains, Infusion from	100	g
Beef Heart, Infusion from	125	g
Bacto Proteose Peptone	5	g
Bacto Neopeptone	5	g
Bacto Dextrose	21	g
Sodium Chloride	2.5	g
Disodium Phosphate	1.25	g
Bacto Agar	15	g

Final pH 7.0 ± 0.2 at 25°C

Precautions

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

Storage

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

User Quality Control

Identity Specifications

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

Solution: 5.9% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, slightly opalescent without significant precipitate.

Reaction of 5.9% Solution at 25°C: pH 7.0 ± 0.2

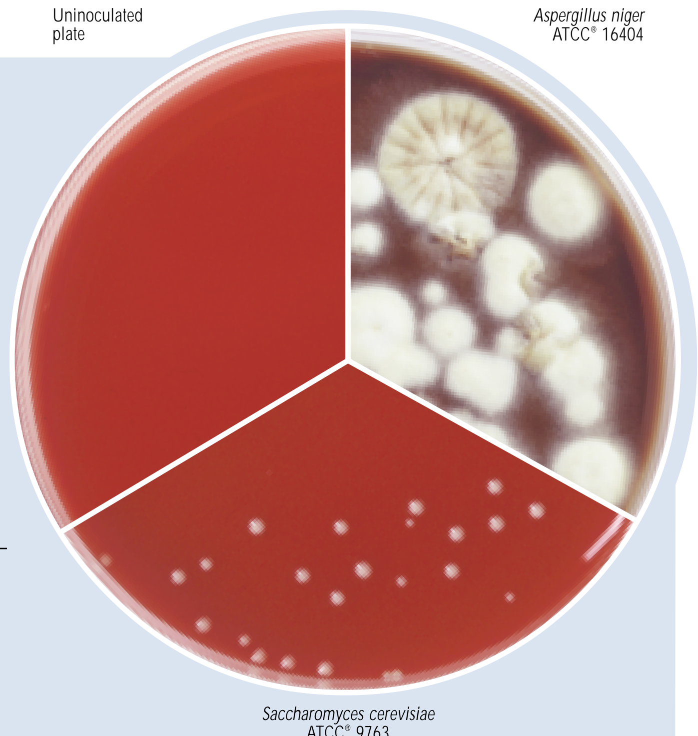
Cultural Response

Prepare SABHI Agar Base according to the label directions and 100 mg/ml of chloromycetin, with and without 10% sheep blood. Inoculate and incubate tubes at 30 ± 2°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH WITH AND WITHOUT BLOOD
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition
Saccharomyces cerevisiae	9763	100-1,000	good
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition
Trichophyton mentagrophytes	9533	100-1,000	good

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

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



Expiration Date

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

Procedure

Materials Provided

SABHI Agar Base

Materials Required but not Provided

Glassware
Autoclave
OPTIONAL: Chloromycetin or other sterile antimicrobics
OPTIONAL: Defibrinated sheep blood

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 15 minutes.
4. Cool medium to 50-55°C.
5. OPTIONAL: To prepare selective medium, aseptically add 1 mL chloromycetin solution (100 mg/ml) to 1 liter of sterile medium.
6. OPTIONAL: To prepare blood agar, aseptically add sterile sheep blood at a concentration of 10% (e.g. 100 ml blood to 900 ml of sterile medium).

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.[6]

Test Procedure

1. Inoculate SABHI tubes/plates with specimen.
2. Incubate SABHI tubes/plates at 30 ± 2°C for up to 7 days.

Results

Observe SABHI tubes/plates for growth and record colony morphology.

Limitations of the Procedure

1. Non-selective fungal media should be used concurrently with selective media when isolating fungi due to the sensitivity of some strains to antibiotics.[5]

References

1. **Sabouraud, R.** 1892. Ann. Dermatol. Syphilol. **3:**1061.
2. **Dixon, D. M., and R. A Fromtling.** 1995. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F .C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Gorman, J. W.** 1967. Sabhi, a new culture medium for pathogenic fungi. Am. J. Med. Technol. **33**:151.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 687-691. Williams & Wilkins, Baltimore, MD.
6. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection and handling, p. 19-32. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken, (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

SABHI Agar Base	500 g	0797-17
	2 kg	0797-07

Bacto® SF Medium

Intended Use

Bacto SF Medium is used for isolating and cultivating fecal streptococci from milk, water, sewage and feces.

Also Known As

Streptococcus Faecalis Medium

Summary and Explanation

Hajna and Perry[1] specified the formulation of SF Broth, a medium that is selective for fecal streptococci when incubated at 45.5°C. SF Broth has been used for testing water and other materials for fecal contamination.[2,3,4] Detection of fecal streptococci is used as an indicator of pollution.

SF medium is used to differentiate Group D enterococci from Group D non-enterococci and other *Streptococcus* spp. that are not Group D. SF Medium is differential in two ways. First, it differentiates based on whether an organism has the ability to grow in the presence of the inhibitor, sodium azide. Second, it detects whether an organism can ferment the carbohydrate, dextrose, producing a pH color change.

Principles of the Procedure

Tryptone is a source of carbon, nitrogen, vitamins and minerals. Dextrose is a fermentable carbohydrate. Sodium Chloride maintains the osmotic balance of the medium. Sodium Azide inhibits cytochrome oxidase of gram-negative bacteria. Brom Cresol Purple is a pH indicator. Phosphates buffer the medium.

Group D enterococci will grow in the presence of azide and ferment glucose. This produces an acid pH that changes the color of the medium from purple to yellow.

Formula

SF Medium

Formula Per Liter

Bacto Tryptone	20 g
Bacto Dextrose	5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g

Sodium Chloride .... 5 g
Sodium Azide .... 0.5 g
Bacto Brom Cresol Purple .... 0.032 g
Final pH 6.9 ± 0.2 at 25°C

Precautions

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

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

Storage

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

Expiration Date

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to gray, may have a light greenish tint, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water. Solution is purple, clear with no precipitate.
Prepared Tubes:	Purple, clear with no precipitate.
Reaction of 3.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare SF Medium per label directions. Inoculate and incubate at 45 ± 0.5°C 18-24 and 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID REACTION
Enterococcus faecalis	19433*	1,000-2,000	good	yellow (acid)
Enterococcus faecium	27270	1,000-2,000	good	yellow (acid)
Escherichia coli	25922*	1,000-2,000	inhibited	no change
Streptococcus bovis	33317	1,000-2,000	none to poor	no change

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

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

Procedure

Materials Provided

SF Medium

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (45 ± 0.5°)

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water. Rehydrate with proportionally less water when liquid inocula will exceed 1 ml.
2. Dispense into tubes with closures.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[1]

1. Inoculate SF Medium with a heavy inoculum from a pure 18-24 hour culture of the test organism.
2. Incubate at 45 ± 0.5°C for 18-48 hours.
3. Read tubes for growth and acid production at 18-24 hours and 40-48 hours.

Results

A positive result is indicated by growth (turbidity) in the medium with the production of a yellowish-brown color (acid production). A negative reaction is indicated by poor or no growth and no color change in the medium.

Limitations of the Procedure

1. Pure cultures of *Streptococcus* spp. should be inoculated into SF Broth.
2. Group D streptococci include both enterococcal and non-enterococcal strains. Consult appropriate references for further identification of Group D streptococci.[5,6,7,8]

References

1. **Hajna, A. A., and C. A. Perry**. 1943. Comparative study of presumptive and confirmative media for bacteria of the coliform group and fecal streptococci. Am. J. Public Health **33**:550-556.
2. **Facklam, R. R.** 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. **23:**1131.
3. **Kenner, B. A., H. F. Clark, and P. W. Kabler.** 1961. Fecal streptococci. I. Cultivation and enumeration of streptococci in surface water. Appl. Microbiol. **9:**15.
4. **Shattock, P. M. F.** *Enterococci*, p. 303-319. *In* J. C. Ayers, A. A. Kraft, H. E. Snyder, and H. W. Walker (eds.), Clinical and biological hazards in food. University Press, Ames, Iowa.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

6. **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.
7. **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.
8. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

SF Medium	500 g	0315-17

SFP Agar

Bacto SFP Agar Base · Bacto Egg Yolk Enrichment 50% Bacto Antimicrobic Vial K · Bacto Antimicrobic Vial P

Intended Use

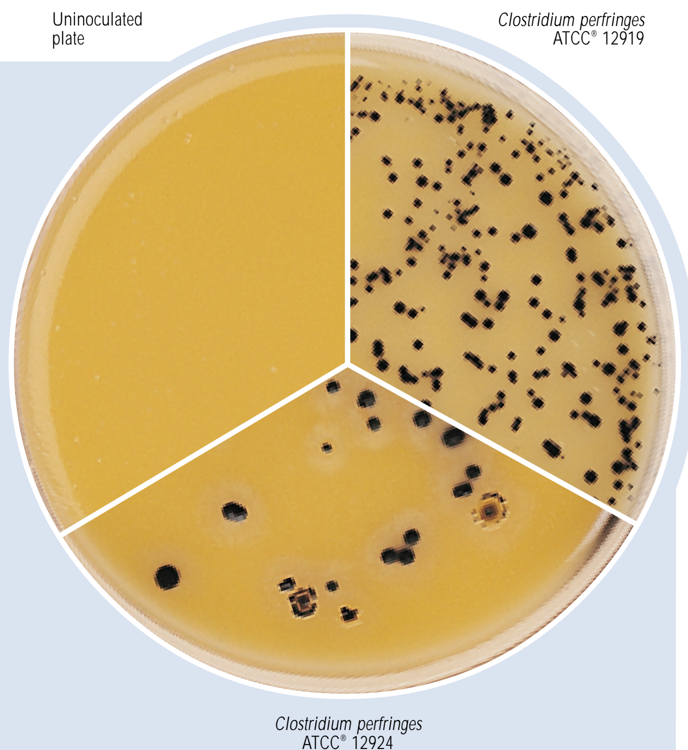
Bacto SFP Agar Base is used with Bacto Egg Yolk Enrichment 50%, Bacto Antimicrobic Vial P and Bacto Antimicrobic Vial K in detecting and enumerating *Clostridium perfringens* in foods.

Also Known As

Tryptose Sulfite Cycloserine (TSC) Agar

Summary and Explanation

Shahidi Ferguson Perfringens (SFP) Agar Base is prepared according to the formulation of Shahidi and Ferguson.[1] With the addition of 50% egg yolk emulsion, both the lecithinase reaction and the sulfite reaction can identify *Clostridium perfringens*. The selectivity of the medium is due to the added kanamycin and polymixin B.



User Quality Control

Identity Specifications

SFP Agar Base

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 4.7% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, slightly opalescent.

Prepared Medium (Final): Canary yellow, opaque.

Reaction of 4.7% Solution at 25°C: pH 7.6 ± 0.2

Egg Yolk Enrichment 50%

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

Antimicrobic Vial K

Dehydrated Appearance: White cake or powder.

Rehydrated Appearance: Colorless, clear solution.

Antimicrobic Vial P

Dehydrated Appearance: White cake or powder.

Rehydrated Appearance: Colorless, clear solution.

Cultural Response

SFP Agar

Prepare the SFP Agar base layer and cover layer per label directions, inoculating the base layer. Incubate at 35 ± 2°C under anaerobic conditions for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR OF COLONIES
Clostridium perfringens	12919	30-300	good	black with halo
Clostridium perfringens	12924	30-300	good	black with halo

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

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

SFP Agar (with added kanamycin and polymixin B) is comparable to Tryptose Sulfite Cycloserine (TSC) Agar, which uses cycloserine as the inhibitory component.[2, 4]

Principles of the Procedure

SFP Agar Base contains Tryptose and Soytone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Ferric Ammonium Citrate and Sodium Sulfite are H_2S indicators. Clostridia reduce sulfite to sulfide, which reacts with iron to form a black iron sulfide precipitate. Antimicrobic Vial P contains Polymyxin B and Antimicrobic Vial K contains Kanamycin; both are inhibitors to organisms other than *Clostridium* spp. Egg Yolk Enrichment 50% provides egg yolk lecithin which some clostridia hydrolyze. Bacto Agar is the solidifying agent.

Formula

SFP Agar Base

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Tryptose	15 g
Bacto Soytone	5 g
Ferric Ammonium Citrate	1 g
Sodium Bisulfite	1 g
Bacto Agar	20 g

Final pH 7.6 ± 0.2 at 25°C

Egg Yolk Enrichment 50%

Sterile concentrated egg yolk emulsion

Antimicrobic Vial K

25,000 mcg Kanamycin per 10 ml vial

Antimicrobic Vial P

30,000 units Polymyxin B per 10 ml vial

Precautions

1. For Laboratory Use.
2. **Antimicrobic Vial K**

   HARMFUL. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   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.

   Antimicrobic Vial P

   MAY BE HARMFUL IF ABSORBED OR INTRODUCED THROUGH SKIN. (US) 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.

   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 SFP Agar Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Egg Yolk Enrichment 50%, Antimicrobic Vial K and Antimicrobic Vial P at 2-8°C.

Expiration Date

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

Procedure

Materials Provided (one of the following)

SFP Agar Base
Egg Yolk Enrichment 50%
Antimicrobic Vial K
Antimicrobic Vial P

Materials Required but not Provided

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

Method of Preparation

SFP Agar Base

Base Layer:

1. Suspend 47 grams 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°C.
4. Add 100 ml Egg Yolk Enrichment 50%, 10 ml of rehydrated Antimicrobic Vial P (30,000 units polymyxin B sulfate) and 4.8 ml rehydrated Antimicrobic Vial K (12 mg kanamycin).
5. Mix thoroughly.

Cover Layer:

1. Suspend 47 grams in 1 liter distilled or deionized water.
2. Prepare as above, except omit Egg Yolk Enrichment 50%.

Egg Yolk Enrichment 50%

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

Antimicrobic Vial K

1. Aseptically add 10 ml sterile distilled or deionized water to the Antimicrobic Vial K.
2. Shake to dissolve contents.

Antimicrobic Vial P

1. Aseptically add 10 ml sterile distilled or deionized water to the Antimicrobic Vial P.
2. Rotate in an end-over-end motion to dissolve contents.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Shahidi, S. A., and A. R. Ferguson.** 1971. New quantitative, qualitative, and confirmatory media for rapid analysis of food for *Clostridium perfringens*. Appl. Microbiol. 21:500-506.
2. **Labbe, R. G., and S. M. Harmon.** 1992. *Clostridium perfringens*, p. 623-635. *In* C. Vanderzant, and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Rhodehamel, E. J., and S. M. Harmon.** 1995. *Clostridium perfringens*, p. 16.01- 16.06. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

SFP Agar Base	500 g	0811-17
Antimicrobic Vial K	6 x 10 ml	3339-60
Antimicrobic Vial P	6 x 10 ml	3268-60
Egg Yolk Enrichment 50%	12 x 10 ml	3347-61
	6 x 100 ml	3347-73

Bacto® SIM Medium

Intended Use

SIM Medium is used for differentiating *Salmonella* and *Shigella* species based on hydrogen sulfide production, indole formation and motility.

Also Known As

Sulfide Indole Motility Medium

Summary and Explanation

Semisolid media have been used extensively in the determination of bacterial motility throughout the history of bacteriology.[1] The production of hydrogen sulfide, indole formation and motility are useful diagnostic tests in the identification of *Enterobacteriaceae*, especially *Salmonella* and *Shigella*. In 1940, Sulkin and Willett[2] showed motility, hydrogen sulfide production and carbohydrate fermentation by members of the *Salmonella* and *Shigella* groups. They called attention to the "brush- like growth" or motility of the typhoid organisms. Green and co-workers[3] used SIM medium to detect motility in a large series of cultures of typhoid organisms.

Principles of the Procedure

Bacto Peptone provides nitrogen, amino acids and additional carbon. Beef Extract is a source of carbon, protein and nutrients. Peptonized Iron and Sodium Thiosulfate are indicators of hydrogen sulfide production. Bacto Agar is a solidifying agent.

Formula

Nitrate Broth

Formula Per Liter

Bacto Peptone	30 g
Bacto Beef Extract	3 g
Peptonized Iron	0.2 g
Sodium Thiosulfate	0.02 g
Bacto Agar	3 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

SIM Medium

Materials Required but not Provided

Glassware

Autoclave
Inoculating needle
SpotTest Indole Reagent Kovacs

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense the medium into tubes to an approximate depth of 3 inches.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Using an inoculum from the growth of a pure culture at 18-24 hours, stab with an inoculating needle two-thirds into the medium. Carefully ensure the needle is withdrawn through the same stab line.
2. Incubate aerobically at 35 ± 2°C for 18-24 hours.
3. Observe for motility, H_2S and Indole production.
4. Add 3-4 drops of SpotTest Indole Reagent Kovacs.

Results

Motility and H_2S production should be determined before the addition of reagents for determination of indole production. Motility is observed as a diffuse growth outward from the stab line or turbidity of the medium. H_2S production is shown by a blackening along the stab line. Indole production is seen as the production of a red color after the addition of 3-4 drops of SpotTest Indole Reagent Kovac's.

Limitations of the Procedure

1. Do not take inoculum from liquid or broth suspensions because growth initiation will be delayed.[4]
2. Reactions are not sufficient to speciate organisms. Additional biochemical and serological tests are required for confirmation.[5]
3. When using Ehrlich's reagent for indole test, 1 ml. of chloroform must be added prior to adding the reagent.[6]

References

1. **Tittsler, R. P., and L. A. Sandholzer.** 1936. The use of semi-solid agar for the detection of bacterial motility. Journal of Bacteriology. **31**:575.
2. **Sulkin and Willett.** 1940. J. Lab. Clin. Med. **25**:649.
3. **Greene, R. A., E. F. Blum, C. T. DeCoro, R. B. Fairchild, M. T. Kaplan, J. L. Landau, and T. R. Sharp.** 1951. Rapid methods for the detection of motility. J. Bact. **62**:347.
4. **Sosa.** 1943. Dr. Carlos G. Malbran. Rev. Inst. Bact. **11**:286.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, p.275-284. vol. 1. Williams & Wilkins, Baltimore, MD.
6. **Koneman, E. W., S. D. Allen, V. R. Dowell, Jr., W. M. Janda, H. M. Sommers, and W. C. Winn, Jr.** 1988. Color Atlas and textbook of diagnostic microbiology, p. 147. 3rd ed. J. B. Lippincott Company, Philadelphia.

Packaging

SIM Medium	500 g	0271-17

User Quality Control

Identity Specifications

Dehydrated Media
Appearance: Beige, homogeneous, free-flowing.

Solution: 3.6% solution, soluble in distilled or deionized water upon boiling. Solution is medium amber, clear to slightly opalescent.

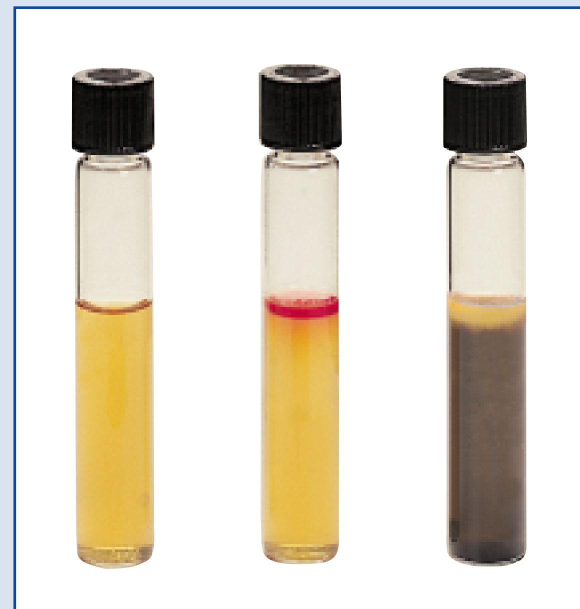
Reaction of 3.6% Solution at 25° C: pH 7.3 ± 0.2

Cultural Response

Prepare SIM Medium per label instructions. Dispense 15 ml of medium into standard size tubes. Inoculate using a straight needle with a single stab to the center through two-thirds of the medium. Incubate tubes at 35 ± 2°C for 18-24 hours and read for growth, H_2S production and motility. Add 3-4 drops of SpotTest™ Indole Reagent Kovacs. Indole production is indicated by a red color after reagent addition.

ORGANISM	ATCC®	GROWTH	H_2S	MOTILITY	INDOLE
Escherichia coli	25922*	good	–	+	+
Salmonella typhimurium	14028*	good	+	+	–
Salmonella typhi	6539	good	+	+	–
Shigella flexneri	12022*	good	–	–	–

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



Uninoculated tube — *Escherichia coli* ATCC® 25922 with indole reagent — *Salmonella typhimurium* ATCC® 14028 with indole reagent

Bacto® SOB Medium

Intended Use

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

Summary and Explanation

SOB Medium was developed by Hanahan[1] as a nutritionally rich growth medium for preparation and transformation of competent cells. Transformation requires making perforations in the bacterium (i.e., making the cells "competent") to allow the introduction of foreign DNA into the cell. To survive this process, competent cells need a rich, isotonic environment.

SOC Medium, used in the final stage of transformation, may be prepared by aseptically adding 20 ml of a filter-sterilized 20% solution of glucose (dextrose) to the sterile SOB Medium. This addition provides a readily available source of carbon and energy in a form *E. coli* can use in mending the perforations and for replication.[2]

Principles of the Procedure

Tryptone and Yeast Extract provide sources of nitrogen and growth factors which allow the bacteria to recover from the stress of transformation and grow well. Sodium Chloride and Potassium Chloride provide a suitable osmotic environment. Magnesium Sulfate is a source of magnesium ions required in a variety of enzymatic reactions, including DNA replication.

Formula

SOB Medium

Formula Per Liter

Bacto Tryptone	20	g
Bacto Yeast Extract	5	g
Sodium Chloride	0.5	g
Magnesium Sulfate, Anhydrous	2.4	g
Potassium Chloride	0.186	g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.8% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 2.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (DH-5)	53868	100-300	Good

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

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 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

SOB Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator 35°C
Waterbath 45-50°C (optional)
Filter-sterilized 20% solution of glucose (dextrose) (optional)

Method of Preparation

1. Dissolve 28 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. If desired, SOC Medium can be prepared by adding 20 ml of a filter-sterilized 20% glucose solution cooled to 45-50°C.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[2]

Results

Growth is evident in the form of turbidity.

References

1. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557.
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

SOB Medium	500 g	0443-17

Bacto® SPS Agar

Intended Use

Bacto SPS Agar is used for detecting and enumerating *Clostridium perfringens* in food.

Also Known As

SPS Agar is also known as Sulfite Polymixin Sulfadiazine Agar or Perfringens Selective Agar.

Summary and Explanation

In the 1950's, Mossel[1] and Mossel et al.[2] proposed media for enumerating anaerobic sulfite-reducing clostridia in foods. Angelotti et al.[3] modified the formula as Sulfite Polymixin Sulfadiazine (SPS) Agar and used it to quantitate *C. perfringens* in foods.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients. Occurrences of food borne illness from *C. perfringens* are usually associated with cooked meat or poultry products.[4] Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.[5] Enumerating the microorganism in food samples plays a role in epidemiological investigation of outbreaks of food borne illness.[4]

Principles of the Procedure

SPS Agar contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Ferric Citrate and Sodium Sulfite are H_2S indicators. Clostridia reduce the sulfite to sulfide which reacts with the iron from ferric citrate to form a black iron sulfide precipitate. Tween® 80 is a dispersing agent. Polymyxin B Sulfate and Sulfadiazine are inhibitors to organisms other than *Clostridium* spp. Sodium Thioglycollate is a reducing agent. Bacto Agar is the solidifying agent.

Formula

SPS Agar

Formula Per Liter

Bacto Tryptone	15 g
Bacto Yeast Extract	10 g
Ferric Citrate	0.5 g
Sodium Sulfite	0.5 g
Sodium Thioglycollate	0.1 g
Tween® 80	0.05 g
Sulfadiazine	0.12 g
Polymyxin B Sulfate	0.01 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

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

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in distilled or deionized water on boiling. Solution light to medium amber, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 7.0 ± 0.2

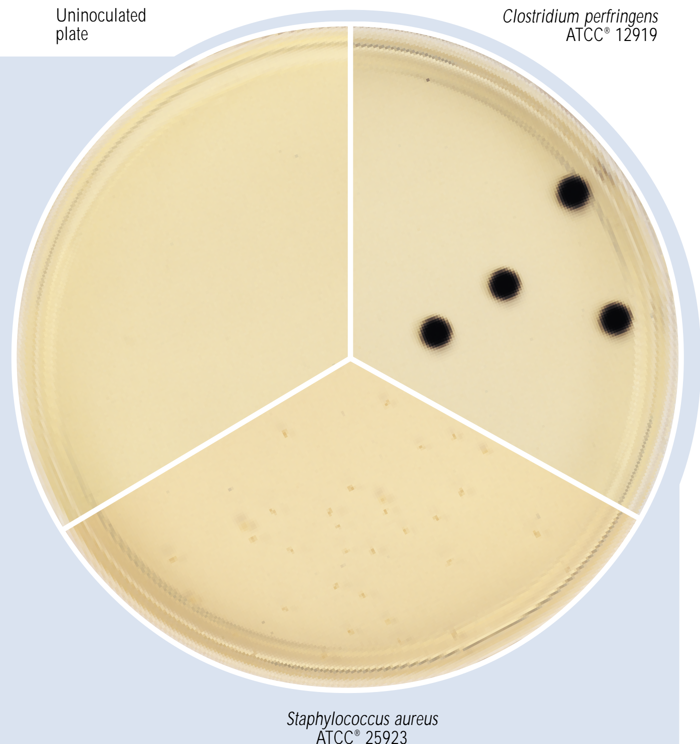
Cultural Response

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

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Clostridium perfringens	12919	100-1,000	good	black colonies
Clostridium sporogenes	11437	100-1,000	none to fair	black colonies
Escherichia coli	25922*	100-1,000	marked to complete inhibition	–
Salmonella typhimurium	14028*	100-1,000	marked to complete inhibition	–
Staphylococcus aureus	25923*	100-1,000	fair to good	white colonies

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

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



Storage

Store the dehydrated medium 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

SPS Agar

Materials Required but not Provided

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

Method of Preparation

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.

Specimen Collection and Preparation

Consult appropriate standard methods.[4, 5.]

Test Procedure

1. Dispense inoculum into sterile Petri dish.
2. Pour medium cooled to 50-55°C over the inoculum.
3. Gently but thoroughly mix the inoculum and medium. Allow to solidify on a flat surface.
4. Incubate anaerobically at 35 ± 2°C for 24-48 hours.

Results

Clostridium perfringens will grow as black colonies with good growth.

Limitations of the Procedure

The high degree of selectivity of SPS Agar may inhibit some strains of *C. perfringens* while other strains that grow may fail to produce distinguishing black colonies.[4]

References

1. **Mossel, R. S**. 1959. Enumeration of sulfite-reducing clostridia occurring in foods. J. Sci. Food Agric. **19**:662.
2. **Mossel, D. A. A., A. S. DeBruin, H. M. J. van Diepen, C. M. A. Vendrig, and G. Zoutewelle.** 1956. The enumeration of anaerobic bacteria, and of *Clostridium* species in particular, in foods. J. Appl. Microbiol. **19**:142.
3. **Angelotti, R., H. E. Hall, M. J. Foster, and K. M. Lewis**. 1962. Quantitation of *Clostridium perfringens* in foods. Appl. Microbiol. **10**:193.
4. **Labbe, R. G., and S. M. Harmon.** 1992. *Clostridium perfringens*, p. 623-635. *In* C. Vanderzant, and D. F. Splittstoesser (ed.),. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Rhodehamel, E. J., and S. M. Harmon**. 1995. *Clostridium perfringens*, p. 16.01- 16.06. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

SPS Agar	100 g	0845-15*
	500 g	0845-17*

*Store at 2-8°C

Bacto® SS Agar

Intended Use

Bacto SS Agar is used for isolating *Salmonella* and some *Shigella*.

Also Known As

SS Agar is also known as *Salmonella-Shigella* Agar.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can produce fatal respiratory, hepatic, splenic, and/or neurological damage.[1] These illnesses result from the consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella.*

Shigella spp. cause classic bacillary dysentery (shigellosis), which is a descending intestinal illness characterized by abdominal pain, fever, and watery diarrhea. *Shigella dysenteriae* can cause a severe form of dysentery that has been reported to have fatality rates of up to 20%. Most cases of shigellosis are individual cases due to person-to-person transmission. When associated with outbreaks, the disease usually is transmitted by contaminated food and/or water.[1]

SS Agar is a modification of the Desoxycholate Citrate Agar described by Leifson.[2] SS Agar was found to be superior to other media for the isolation of *Salmonella* and *Shigella* spp.[3] Ewing and Bruner found SS Agar to have the advantage that large amounts of inoculum could be used when isolating *Salmonella* or *Shigella* from clinical samples.[4] Caudill[5] reported on the satisfactory use of SS Agar in isolation of *Shigella* organisms. Hormaeche and his co-workers[6] used SS Agar with other media for isolation of *Shigella* as the causative agent of infantile summer diarrhea.

The use of SS Agar is recommended for testing clinical specimens for the presence of *Salmonella* and some *Shigella* spp.[1,7] For food testing, consult appropriate references on the use of SS Agar.[8]

Principles of the Procedure

In SS Agar, Bacto Bile Salts No. 3 and Brilliant Green are complementary in inhibiting gram-positive bacteria, most coliform bacteria, and the swarming phenomenon of *Proteus* spp., while allowing

Salmonella spp. to grow. Sodium thiosulfate and ferric citrate allow the detection of hydrogen sulfide by the production of colonies with black centers. Lactose is the carbohydrate present in SS Agar. Neutral red and brilliant green are present as pH indicators.

Formula

SS Agar

Formula Per Liter

Bacto Beef Extract	5	g
Bacto Proteose Peptone	5	g
Bacto Lactose	10	g
Bacto Bile Salts No. 3	8.5	g
Sodium Citrate	8.5	g
Sodium Thiosulfate	8.5	g
Ferric Citrate	1	g
Bacto Agar	13.5	g
Brilliant Green	0.33	mg
Neutral Red	0.025	g

Final pH 7.0 ± 0.2 at 25°C

Precautions

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

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

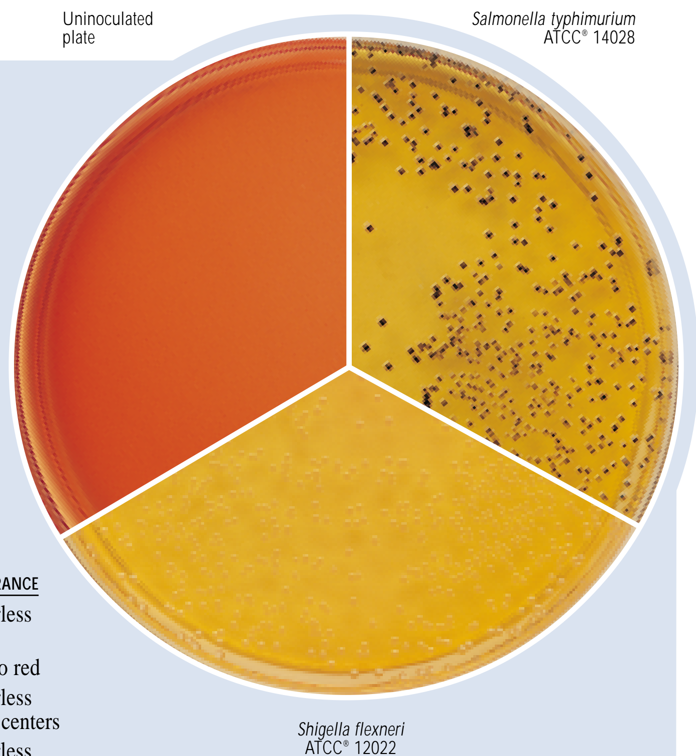
SS Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 60 grams in 1 liter distilled or deionized water.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light buff to pink, free flowing, homogeneous.
Solution:	6.0% solution, soluble in distilled or deionized water on boiling. Solution is red-orange, very slightly to slightly opalescent.
Prepared Plates:	Red-orange, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare SS Agar per label directions. Inoculate and incubate plates at 35 ± 2°C for 18-24 hours and 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1,000-2,000	partial inhibition	colorless
Escherichia coli	25922*	1,000-2,000	partial inhibition	pink to red
Salmonella typhimurium	14028*	100-1,000	good	colorless w/black centers
Shigella flexneri	12022*	100-1,000	fair to good	colorless

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

2. Heat to boiling for no more than 2-3 minutes to dissolve completely. Avoid overheating. DO NOT AUTOCLAVE.
3. Cool to 45-50°C in a waterbath.
4. Dispense into sterile Petri dishes. Allow the surface of the medium to air dry for two hours by leaving the lids ajar.

Specimen Collection and Preparation

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

Test Procedure

For isolation of *Salmonella* and *Shigella* spp. from clinical specimens, inoculate fecal samples and rectal swabs onto one quadrant of a SS Agar plate and streak for isolation. This will permit the development of discreet colonies. Incubate plates at 35°C. Examine at 24 hours and again at 48 hours for colonies resembling *Salmonella* or *Shigella* spp. Note: SS Agar is inhibitory to some strains of *Shigella* spp. For additional information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[1,7]

For testing food samples, consult appropriate references.[8]

Results

Enteric organisms are differentiated by their ability to ferment lactose. *Salmonella* and *Shigella* spp. are lactose non-fermenters and form colorless colonies on SS Agar. *Salmonella* spp. that are H_2S positive produce colonies with black centers. Some *Shigella* spp. are inhibited on SS Agar.

Coliforms are partially inhibited on SS Agar. *E. coli* produces pink to red colonies and may have some bile precipitation. Colonies of *Enterobacter aerogenes* appear cream to pink in color. *Citrobacter* and *Proteus* spp. may grow on SS Agar and produce colonies with gray to black centers due to H_2S production. *Enterococcus faecalis* is partially inhibited on SS Agar; colonies of *E. faecalis* are colorless.

Limitations of the Procedure

1. SS Agar is a highly selective medium. For this reason, it is not recommended as the sole medium for primary isolation of *Shigella*.[1,2,9] Some strains of *Shigella* may not grow.
2. A few nonpathogenic organisms may grow on SS Agar. These organisms can be differentiated by their ability to ferment lactose.[10]

References

1. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella* and *Yersinia*, p. 450-456. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. **40:**581.
3. **Rose, H. M., and M. H. Kolodny.** 1942. The use of SS (*Shigella-Salmonella*) Agar for the isolation of Flexner Dysentery bacilli from the feces. J. Lab. Clin. Med. **27:**1081-1083.
4. **Ewing, W. H., and D. W. Bruner.** 1947. Selection of *Salmonella* and *Shigella* cultures for serologic classification. Am. J. Clin. Pathol. **17:**1-12.
5. **Caudill, F. W., R. E. Teague, and J. T. Duncan.** 1942. A rural shiga dysentery epidemic. JAMA **119:**1402-1406.
6. **Hormaeche, E., N. L. Surraco, C. A. Peluffo, and P. L. Aleppo.** 1943. Causes of infantile summer diarrhea. Am. J. Dis. Child. **66:**539-551.
7. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.1-1.20.47. *In* Isenberg, H. D. (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
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.
9. **Taylor, W. I., and B. Harris.** 1965. Isolation of shigellae. II. Comparison of plating media and enrichment broths. Am. J. Clin. Pathol. **44:**476.
10. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

SS Agar	100 g	0074-15
	500 g	0074-17
	2 kg	0074-07
	10 kg	0074-08

Sabouraud Media

Bacto® Sabouraud Agar Modified · Bacto Sabouraud Dextrose Agar Sabouraud Dextrose Broth · Bacto Sabouraud Maltose Agar Bacto Sabouraud Maltose Broth · Bacto Fluid Sabouraud Medium

Intended Use

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

Bacto Sabouraud Dextrose Agar and Broth and Bacto Sabouraud Maltose Agar and Broth are used for culturing yeasts, molds and aciduric microorganisms.

Bacto Fluid Sabouraud Medium is used for cultivating yeasts, molds and aciduric microorganisms and for detecting yeasts and molds in normally sterile materials.

User Quality Control

Identity Specification

Sabouraud Agar Modified

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

Solution: 5.0% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent without significant precipitate.

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

Reaction of 5.0% Solution at 25°C: pH 7.0 ± 0.2

Sabouraud Dextrose Agar

Dehydrated Appearance: Light 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 without significant precipitate.

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

Reaction of 6.5% Solution 25°C: pH 5.6 ± 0.2

Sabouraud Dextrose 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 5.6 ± 0.2

Sabouraud Maltose Agar

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

Solution: 6.5% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent, may have a slight precipitate.

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

Reaction of 6.5% Solution at 25°C: pH 5.6 ± 0.2

Sabouraud Maltose Broth

Dehydrated Appearance: White, free-flowing, homogeneous.

Solution: 5.0% solution, soluble in distilled or deionized water. Solution is light amber, clear to slightly opalescent.

Prepared Medium: Light amber, clear to slightly opalescent.

Reaction of 5.0% Solution at 25°C: pH 5.6 ± 0.2

continued on following page

Summary and Explanation

Sabouraud Agar Modified is a modification of the Sabouraud Dextrose Agar formulation devised by Raymond Sabouraud for his dermatophyte studies.[1] Sabouraud Agar Modified, used for the recovery of dermatophytes[2], contains reduced dextrose (2%) and has a neutral pH (7.0). The selectivity of the medium can be improved with the addition of antibiotics, such as chloramphenicol to inhibit bacterial growth and cycloheximide to inhibit saprophytic fungi.[3,4]

Sabouraud Dextrose Agar and Sabouraud Dextrose Broth are modifications of the Dextrose Agar described by Sabouraud.[1] They are used for cultivating pathogenic fungi, particularly those associated with skin infections. The high dextrose concentration and acidic pH make these media selective for fungi.[5] Georg[6] demonstrated that the addition of cycloheximide, streptomycin and penicillin to Sabouraud Dextrose Agar produces an excellent medium for the primary isolation of dermatophytes. Sabouraud Dextrose Agar is also used for determining the microbial content of cosmetics[7] and for the mycological evaluation of food.[8] Sabouraud Dextrose Agar is available in the dehydrated form and prepared in 200 ml amounts. In the prepared form, Sabouraud Dextrose Agar is used for pouring plates.

Sabouraud Maltose Agar is a modification of Sabouraud Dextrose Agar with maltose substituted for dextrose. It is a selective medium due to the acid pH. Davidson, Dawding and Buller[9] reported that Sabouraud Maltose Agar was a satisfactory medium in their studies of the infections caused by *Microsporon audouini*, *M. lanosum* and *Trichophyton gypseum*. Davidson and Dawding[10] also used this medium in isolating *T. gypseum* from a case of tinea barbae.

Sabouraud Maltose Broth is a modification of Sabouraud Dextrose Broth in which maltose is substituted for dextrose. It is selective due to its acid pH and is used for the detection of fungi.

Fluid Sabouraud Medium is employed in sterility test procedures for determining the presence of molds, yeasts and aciduric microorganisms. The acid reaction of the final medium is inhibitive to a large number of bacteria and makes the medium particularly well suited for cultivating fungi and acidophilic microorganisms.

Principles of the Procedure

Sabouraud Agar Modified, Sabouraud Dextrose Agar**,** and Sabouraud Dextrose Broth contain Neopeptone which provides the carbon and nitrogen required for growth of a wide variety of organisms. Dextrose is included as an energy source. Bacto Agar is incorporated into the agar media as a solidifying agent.

Sabouraud Maltose Agar and Sabouraud Maltose Broth contain Neopeptone which provides the carbon and nitrogen sources required for growth of a wide variety of organisms. Maltose is included in the medium as an energy source. Sabouraud Maltose Agar contains Bacto Agar as the solidifying agent.

Fluid Sabouraud Medium contains Casitone and Peptamin which provide nitrogen, vitamins, minerals and amino acids. Dextrose is an energy source.

Formula

Sabouraud Agar Modified

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Dextrose	20 g
Bacto Agar	20 g

Final pH 7.0 ± 0.2 at 25°C

Sabouraud Dextrose Agar

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Dextrose	40 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Sabouraud Dextrose Broth

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Dextrose	20 g

Final pH 5.6 ± 0.2 at 25°C

Sabouraud Maltose Agar

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Dextrose	40 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Sabouraud Maltose Broth

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Maltose	40 g

Final pH 5.6 ± 0.2 at 25°C

Fluid Sabouraud Medium

Formula Per Liter

Bacto Casitone	5 g
Bacto Peptamin	5 g
Bacto Dextrose	20 g

Final pH 5.7 ± 0.2 at 25°C

Precautions

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

Storage

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

Store prepared Sabouraud Dextrose Agar 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.

User Quality Control cont.

Fluid Sabouraud Medium

Dehydrated Appearance: Off-white, free-flowing, homogeneous.

Solution: 3.0% solution, soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.

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

Reaction of 3.0% Solution at 25°C: pH 5.7 ± 0.2

Cultural Response

Sabouraud Agar Modified, Sabouraud Maltose Agar, Sabouraud Maltose Broth, Fluid Sabouraud Mediumm Sabouraud Dextrose Agar, Sabouraud Dextrose Broth

Prepare dehydrated medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

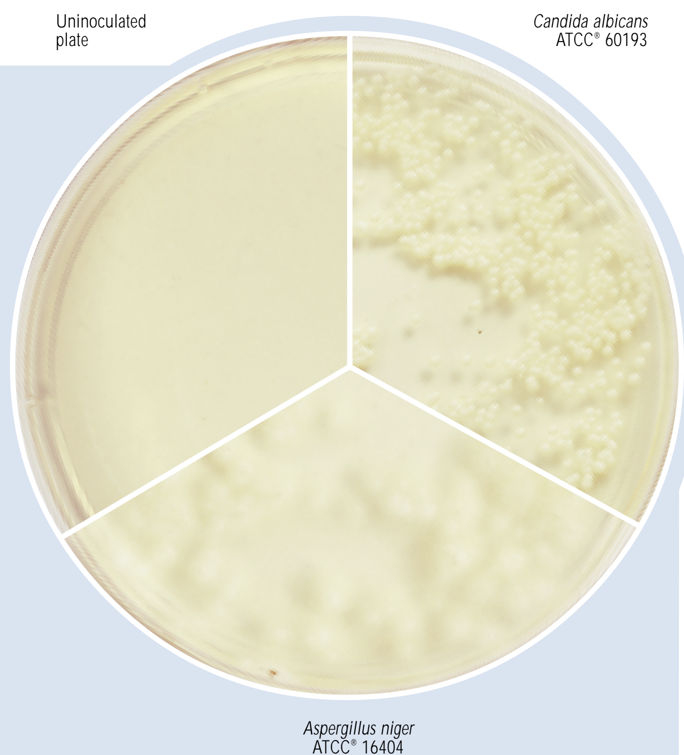
ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

Sabouraud Dextrose Agar (prepared)

Melt medium and aseptically dispense into plates. Inoculate and incubate at 30 ± 2°C for 18-48 hours, except *Aspergillus niger* which is incubated at room temperature for 3-5 days.

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

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



Procedure

Materials Provided

Sabouraud Agar Modified
Sabouraud Dextrose Agar (dehydrated or prepared)
Sabouraud Dextrose Broth
Sabouraud Maltose Broth
Sabouraud Maltose Agar
Fluid Sabouraud Medium. (For Laboratory Use)

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Sterile Petri dishes or tubes with closures
Waterbath (optional)

Method of Preparation

Dehydrated Media

1. Suspend the indicated amount of dehydrated medium in 1 liter of distilled or deionized water and boil to dissolve completely. Avoid overheating which could cause a softer medium.
   Sabouraud Agar Modified - 50 grams
   Sabouraud Dextrose Agar - 65 grams
   Sabouraud Maltose Agar - 65 grams
   Dissolve the indicated amount of dehydrated medium in 1 liter of distilled or deionized water.
   Sabouraud Dextrose Broth - 30 grams
   Sabouraud Maltose Broth - 50 grams
   Fluid Sabouraud Medium - 30 grams
2. Autoclave at 121°C for 15 minutes.

Prepared Sabouraud Dextrose Agar

Melt the agar to pour into plates by one of the following methods.

- Loosen the bottle caps, then autoclave bottles at 121°C for 3 minutes to melt the agar. A small solidified mass may remain that can be melted by swirling the hot agar. Autoclave time depends on the number of bottles in the chamber.
  NOTES: Autoclave small batches to limit darkening of the medium.

Long cycles have a tendency to shrink the clear label material.

- Heat bottles in boiling water. Time will vary; it may take up to 40 minutes to melt the agar.
- Microwave the bottles to melt the agar. Time will vary with the microwave and the number of bottles to be melted. When microwaving, boiling over is a significant problem with smaller bottles.

Specimen Collection and Preparation

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

Test Procedure

Consult appropriate references for recommended test procedures.[2]

Results

Growth is evident in the form of turbidity.

Limitations of the Procedure

1. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi.
2. Avoid overheating a medium with an acidic pH because this often causes a soft medium.

References

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Packaging

Sabouraud Agar Modified	500 g	0747-17
	2 kg	0747-07
Sabouraud Dextrose Agar	50 g	0109-17
	100 g	0109-15
	10 kg	0109-08
	2 kg	0109-07
	10 x 200 ml	9074-76
Sabouraud Dextrose Broth	500 g	0382-17
	100 g	0382-15
	2 kg	0382-07
Sabouraud Maltose Agar	500 g	0110-17
	2 kg	0110-07
Sabouraud Maltose Broth	500 g	0429-17
Fluid Sabouraud Medium	500 g	0642-17

Bacto® Schaedler Agar
Bacto Schaedler Broth

Intended Use

Bacto Schaedler Agar is used with or without blood in cultivating and enumerating anaerobic and aerobic microorganisms.

Bacto Schaedler Broth is used for cultivating anaerobic and aerobic microorganisms with or without added blood or enrichment.

Summary and Explanation

Schaedler Agar and Schaedler Broth are prepared according to the formulation described by Schaedler, Dubos and Costello[1] and modified by Mata, Carrillo and Villatoro.[2] Modifications include reduced dextrose to avoid interference with hemolytic reactions and reduced yeast extract to avoid darkening of the medium[3] as well as adjusted sodium chloride and peptone concentrations. Schaedler Broth is the same formulation as Schaedler Agar but with the agar omitted.

While studying the gastrointestinal flora of mice, Schaedler et al.[1] formulated a medium to recover both aerobic and anaerobic microorganisms. Mata et al.[2] used a modification of the Schaedler formula to study human fecal microflora.

Stalons, Thornsberry and Dowell[4] evaluated nine broth media in varied carbon dioxide atmospheres for their ability to support growth of anaerobic bacteria. Schaedler Broth in an atmosphere of 5% CO_2, 10% hydrogen and 85% nitrogen exhibited the fastest and highest growth response.

Anaerobic bacteria cause a variety of human infections including endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.[5,6] Since anaerobes vary in their sensitivity to oxygen and nutritional requirements[7], appropriate collection, culture medium and incubation are vital to recovery.[7] Schaedler media are suitable for standard procedures used in cultivating anaerobic bacteria.[7,8,9]

User Quality Control

Identity Specifications

Schaedler Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.19% solution, soluble in distilled or deionized water on boiling. Light to medium amber, clear to slightly opalescent, may have a fine precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent, may have a fine precipitate.
Reaction of 4.19% Solution at 25°C	pH 7.6 ± 0.2

Schaedler Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.84% solution, soluble in distilled or deionized water on boiling 1-2 minutes. Light to medium amber, clear to slightly opalescent, may have a very slight black precipitate.
Prepared Medium:	Light to medium amber, clear to very slightly opalescent, may have a very slight black precipitate.
Reaction of 2.84% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Prepare Schaedler Agar or Schaedler Broth per label directions. Prereduce Schaedler Broth prior to inoculation with anaerobic organisms. Inoculate medium; incubate at 35 ± 2°C for 18-48 hours under aerobic or anaerobic conditions, depending on the requirements of the inoculum.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis†	25285*	100-1,000	good
Bacteroides vulgatus†	8482	100-1,000	good
Clostridium novyi B†	27606	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.

†Incubate anaerobically.

Principles of the Procedure

Tryptic Soy Broth, Proteose Peptone No.3 and Yeast Extract provide the vitamins, nitrogen and amino acids in Schaedler media. Dextrose is a carbon source, and Tris (Hydroxymethyl) Amino Methane is used to buffer the medium. Hemin (X factor) stimulates growth. Bacto Agar is the solidifying agent in Schaedler Agar.

The following supplements can be added to Schaedler media.

- Sheep, horse or rabbit blood (5%) - for enrichment and for detecting hemolysis and pigment production.[9]
- Vitamin K_1 (1%) - to promote growth of some pigmented *Prevotella* and *Porphyromonas* spp. (formerly known as *Bacteroides*).[9]
- Colistin and nalidixic acid (0.01 grams/liter, each) (Schaedler CNA agar) - for selectively isolating anaerobic gram-positive cocci.[10]
- Kanamycin (0.01 grams/liter) and vancomycin (7.5 mg/liter) (Schaedler KV Agar) - for selectively isolating anaerobic gram-negative bacteria.[10]

Formula

Schaedler Agar

Formula Per Liter

Bacto Tryptic Soy Broth	10 g
Bacto Proteose Peptone No.3	5 g
Bacto Yeast Extract	5 g
Bacto Dextrose	5 g
Tris (Hydroxymethyl) Amino Methane	3 g
L-Cystine	0.4 g
Hemin	0.01 g
Bacto Agar	13.5 g

Final pH 7.6 ± 0.2 at 25°C

Schaedler Broth

Formula Per Liter

Bacto Tryptic Soy Broth	10 g
Bacto Proteose Peptone No.3	5 g
Bacto Yeast Extract	5 g

Bacto Dextrose	5 g
Tris (Hydroxymethyl) Amino Methane	3 g
L-Cystine	0.4 g
Hemin	0.01 g

Final pH 7.6 ± 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

Schaedler Agar
Schaedler Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes
Sterile defibrinated sheep, horse or rabbit blood (optional)

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:
   Schaedler Agar - 41.9 grams/liter;
   Schaedler Broth - 28.4 grams/liter.
2. OPTIONAL: Add 1 ml of 1% vitamin K_1 in absolute ethanol.
3. Heat to boiling for 1-2 minutes to dissolve completely.
4. Autoclave at 121°C for 15 minutes. Cool to room temperature.
5. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.

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 techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion of aerobic and anaerobic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[7,8,9] For the examination of bacteria in food, refer to standard methods.[11,12,13]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[7]
3. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[7]
4. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure that the organism is an anaerobe.[7]
5. Because of the high dextrose concentration in Schaedler Agar when it is supplemented with 5% blood, beta-hemolytic streptococci may produce a hemolytic reaction that is similar to alpha hemolysis.

References

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Packaging

Schaedler Agar	500 g	0403-17
Schaedler Broth	500 g	0534-17

Bacto® Selenite Broth

Intended Use

Bacto Selenite Broth is used for enriching *Salmonella* spp. during isolation procedures and for isolating *Salmonella* in foods.

Also Known As

Selenite Broth is also referred to as Selenite F (Fecal) Broth.

Summary and Explanation

Selenite Broth is used as a selective enrichment for the cultivation of *Salmonella* spp. that may be present in small numbers and competing with intestinal flora. *Salmonella* organisms are also injured in food-processing procedures, including exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers.[1] Although injured cells may not form colonies on selective media, they can cause infection if ingested.[2] *Salmonella* spp. 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]

The formula of Selenite Broth is described by Leifson[4] as Selenite F Broth. Guth,[5] according to Handel and Theodorascu, observed that *Escherichia coli* was more susceptible to the toxicity of sodium selenite than *S. typhi.* Guth employed sodium selenite as a selective agent in agar medium and enrichment broth for the isolation of *S. typhi* from feces. Leifson[4] extended Guth's observations and developed a selenite agar and selenite broth for the isolation of typhoid and paratyphoid bacilli from clinical specimens.

Leifson[4] found the selenite broth was not sufficiently toxic to completely inhibit fecal coliforms and enterococci. These organisms were inhibited during the first 8 -12 hours but increased rapidly after this time period. *Salmonella* spp. multiply fairly rapidly after inoculation. It is suggested that selenium toxicity may be a reaction with sulphur and sulphydryl groups in certain strains of bacteria.[6,7]

There have been many modifications of Selenite Broth from the original formula described by Leifson. Selenite Cystine Broth is used as a selective enrichment broth recommended by AOAC[8] and USP[9] for detecting *Salmonella* in food, dairy products and other materials of sanitary importance. Selenite Brilliant Green Sulfa (SBS) Enrichment Broth and Selenite Brilliant Green Mannitol (SBM) Enrichment Broth have also been used for the cultivation of *Salmonella*.[10]

Selenite Broth conforms with APHA[11] and is specified in Clinical Microbiology Procedures Handbook[12] and Manual of Clinical Microbiology.[13]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in distilled or deionized water on boiling; very light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Medium:	Very light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Selenite Broth per label directions. Incubate inoculated medium at 35 ± 2°C for 18-24 hours. After incubation, subculture onto MacConkey Agar plates and incubate plated media at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	CFU	GROWTH	MACCONKEY AGAR
Escherichia coli	25922*	100-1,000	partial to marked inhibition	pink w/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 as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Bacto Tryptone provides the nitrogen, vitamins and amino acids in Selenite Broth. Bacto Lactose is a fermentable carbohydrate. Selenite is reduced by organism growth. A rise in pH decreases the selective activity of the selenite. The acid produced by lactose fermentation helps to maintain a neutral pH. Sodium selenite inhibits the growth of gram-positive bacteria and many gram-negative bacteria. Sodium phosphate is a buffering agent.

Formula

Selenite Broth

Formula Per Liter

Bacto Tryptone	5 g
Bacto Lactose	4 g
Sodium Selenite	4 g
Sodium Phosphate	10 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Very TOXIC.** FATAL IF INHALED OR SWALLOWED.(US) VERY TOXIC BY INHALATION AND IF SWALLOWED.(EC) DANGER OF CUMULATIVE EFFECTS.(EC) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Kidneys, Spleen, Liver.

   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 skin irritation persists, seek medical advice. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.

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

Storage

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

Expiration Date

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

Procedure

Material Provided

Selenite Broth

Materials Required But Not Provided

Glassware
Distilled or deionized water
Incubator
Waterbath (45-50°C) (optional)
Sterile tubes

Method of Preparation

1. Dissolve 23 grams in 1 liter distilled or deionized water.
2. Heat to boiling to pasteurize.
3. Avoid overheating. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

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

Test Procedure

For a complete discussion on the isolation and identification of *Salmonella* species refer to the 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.
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12. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington. D.C.
13. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.)** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.

Packaging

Selenite Broth	100 g	0275-15
	500 g	0275-17
	10 kg	0275-08

Bacto® Selenite Cystine Broth

Intended Use

Bacto Selenite Cystine Broth is used for selectively enriching *Salmonella* in food and water.

Summary and Explanation

Selenite Cystine Broth is the formulation by Leifson[1] with cystine added. Leifson determined that Selenite Broth favored the growth of *Salmonella* while reducing growth of fecal coliforms and enterococci.[1]

The growth and recovery of *Salmonella* in food samples can be hindered by non-*Salmonella* bacteria, substances indigenous to the food sample, and in dried, processed food, the *Salmonella* may be present in low numbers and in a injured condition.[2] Using protocols that involve preenrichment, selective enrichment and selective plating increase the likelihood of recovering *Salmonella*. In most standard method procedures Selenite Cystine Broth is recommended in the selective enrichment step.[2,3,4,5,6] As a selective enrichment medium, Selenite

Cystine Broth is formulated to allow the proliferation of *Salmonella* and while inhibiting the growth of competing non-*Salmonella* bacteria.[2]

Principles of the Procedure

Selenite Cystine Broth contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Lactose is the carbohydrate. Sodium Acid Selenite inhibits gram-positive bacteria and most enteric gram-negative bacteria except *Salmonella*. L-cystine is a reducing agent.

Formula

Selenite Cystine Broth

Formula Per Liter

Bacto Tryptone	5 g
Bacto Lactose	4 g
Disodium Phosphate	10 g
Sodium Acid Selenite	4 g
L-Cystine	0.01 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **VERY TOXIC.** FATAL IF INHALED OR SWALLOWED. (US) VERY TOXIC BY INHALATION AND IF SWALLOWED. (EC) DANGER OF CUMULATIVE EFFECTS. (EC) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   TARGET ORGAN(S): Lungs, Kidneys, Spleen, Liver.

   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 skin irritation persists, seek medical advice. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in distilled or deionized water on boiling.
Prepared Medium:	Very light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Selenite Cystine Broth per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 24 ± 2 hours and subculture on MacConkey Agar plates.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	100-1,000	partial to complete inhibition	pink with bile precipitate
Salmonella typhimurium	14028*	100-1,000	good	colorless
Shigella sonnei	9290*	100-1,000	fair to good	colorless

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

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

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

Selenite Cystine Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Tetrathionate Broth
Bismuth Sulfite Agar
XLD Agar
Hektoen Enteric Agar
MacConkey Agar

Method of Preparation

1. Suspend 23 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes to a depth of 60 mm.
4. DO NOT AUTOCLAVE. Use immediately.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure[4,5]

1. Prepare sample according to food type.
2. Inoculate into recommended pre-enrichment broth.
3. Transfer 1 ml of mixture to 10 ml Selenite Cystine Broth and to 10 ml Tetrathionate Broth.
4. Incubate at 35°C for 24 ± 2 hours.
5. Mix and streak 3 mm loopful (10 µl) of sample from both broths onto Bismuth Sulfite Agar, Xylose Lysine Desoxycholate Agar, Hektoen Enteric Agar or MacConkey Agar.
6. Incubate plates at 35°C for 24 ± 2 hours.
7. Examine plates for the presence of colonies that are typical for *Salmonella* spp.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. A brick red precipitate may appear if Selenite Cystine Broth is overheated during preparation or exposed to excessive moisture during storage.

References

1. **Leifson, E**. 1936. New selenite selective enrichment medium for the isolation of typhoid and paratyphoid (*Salmonella*) bacilli. Am J. Hyg. **24**:423-432.
2. **Flowers, R. S., J-Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Flowers, R. S., W. H. Andrews, E. W. Donnely, and E. Koenig.** 1992. Pathogens in milk and milk products, p. 103-124. *In* R. T. Marshall (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **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.
5. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
6. **United States Pharmacopeial Convention**. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.

Packaging

Selenite Cystine Broth	100 g	0687-15
	500 g	0687-17
	2 kg	0687-07
	10 kg	0687-08

Bacto® Simmons Citrate Agar

Intended Use

Bacto Simmons Citrate Agar is used for differentiating *Enterobacteriaceae* based on citrate utilization.

Summary and Explanation

Koser[1] first developed a liquid medium for differentiating coliforms from fecal coliforms. Fecal coliforms were unable to use citrate as the sole source of carbon and inorganic ammonium salt as a sole source of nitrogen. Non-fecal coliforms, such as *Enterobacter aerogenes* or *Salmonella enteritidis* could use citrate in such a medium with resultant alkalinity. The liquid medium had the disadvantage of appearing turbid when large inocula were used although no growth had taken place. This observation led Simmons[2] to devise a solid medium that eliminated the problem with turbidity.

Simmons Citrate Agar is a modification of Koser's medium to which brom thymol blue and 1.5% agar have been added. Organisms able to metabolize the citrate grow luxuriantly. The medium is alkalinized and changes from its initial green to deep blue in 24-48 hours. *E. coli* either do not grow at all on this medium, or grow so sparsely that no change in reaction is apparent.

Simmons Citrate Agar is recommended for differentiation of enteric gram-negative bacilli from clinical specimens,[3,4] water samples,[5] and food samples.[6-9]

Principles of the Procedure

The ammonium dihydrogen phosphate is the sole source of nitrogen in Simmons Citrate Agar. Magnesium is a cofactor for a variety of metabolic reactions. Phosphate acts as a buffer. Sodium citrate is the sole source of carbon in this medium. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. Brom thymol blue is the pH indicator. Organisms that can utilize ammonium dihydrogen phosphate and sodium citrate as their sole sources of nitrogen and carbon will grow on this medium and produce a color change from green (neutral) to blue (alkaline).

Formula

Simmons Citrate Agar

Formula Per Liter

Magnesium Sulfate	0.2 g
Ammonium Dihydrogen Phosphate	1 g
Dipotassium Phosphate	1 g
Sodium Citrate	2 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Brom Thymol Blue	0.08 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

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

Storage

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

Simmons Citrate Agar

Materials Required But Not Provided
Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Tubes with closures
Autoclave
Incubator (35°C)

Method of Preparation
1. Suspend 24.2 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 a slanted position with long slant and short butt.

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.[3-9]
2. Process each specimen, using procedures appropriate for that specimen or sample.[3-9]

Test Procedure
1. Obtain a pure culture of the organism to be tested.
2. With an inoculating needle or loop, pick the center of a well-isolated colonies obtained from solid culture media.
3. Streak only the surface of the slant with a light inoculum.
4. Loosen the closure on the tube.
5. Incubate at 35 ± 2°C for 18-48 hours.

Results
A positive reaction is indicated by growth on the slant with an intense blue color (alkaline reaction). A negative reaction is indicated by no growth to poor growth without change in color (medium remains green).

Limitations of the Procedure
1. When inoculating a variety of biochemicals, flame the inoculating loop or needle before streaking Simmons Citrate Agar or inoculate Simmons Citrate Agar first to avoid a false positive result.[10]
2. Some citrate positive organisms require 48 hours or longer incubation for a pH change to occur.[10]

References

1. **Koser, S. A.** 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bacteriol. **8:**493.
2. **Simmons, J. S.** 1926. A culture medium for differentiating organisms of typhoid- colon aerogenes groups and for isolation of certain fungi. J. Infect. Dis. **39:**209.
3. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.0-1.20.47. *In* Isenberg, H.D. (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. **Baron, E. J., L. R. Peterson, S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

User Quality Control

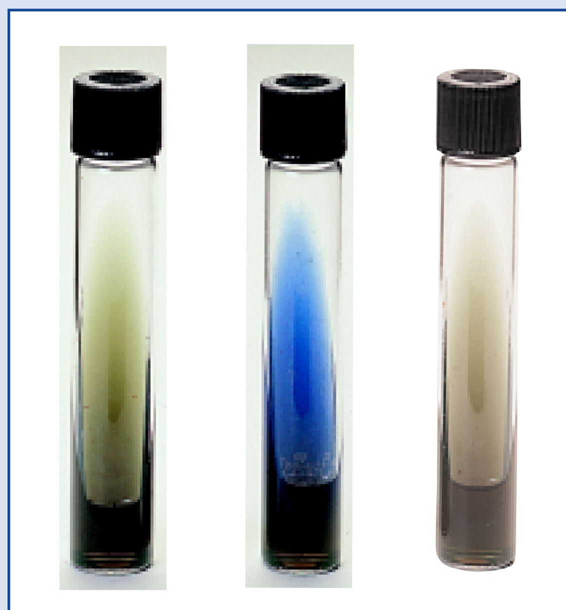
Identity Specifications

Dehydrated Appearance:	Mustard yellow to yellow-green, free flowing, homogeneous.
Solution:	2.42% solution; soluble in distilled or deionized water on boiling. Solution is forest green, slightly opalescent, may have a slight precipitate.
Prepared Tubes:	Forest green, slightly opalescent, may have a slight precipitate.
Reaction of 2.42% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response
Prepare Simmons Citrate Agar per label directions. Inoculate with 1 µl of a dilution equivalent to a 0.5 McFarland Standard and incubate the tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	COLONY COLOR
Enterobacter aerogenes	13048*	good	blue
Escherichia coli	25922*	none to poor	green
Salmonella typhimurium	14028*	good	blue

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



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

6. **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.
7. **FDA Bacteriological Analytical Manual**, 8th ed. AOAC International, Gaithersburg, MD.
8. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
9. **Federal Register.** 1996. Pathogen reduction; hazard analysis and critical point (HACCP) systems; final rule. Fed. Regis. **61:**38917-38925.
10. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Simmons Citrate Agar	100 g	0091-15
	500 g	0091-17

Bacto® Skim Milk

Intended Use

Bacto Skim Milk is used for preparing microbiological culture media and for differentiating organisms based on coagulation and proteolysis of casein.

Summary and Explanation

Skim Milk is soluble, spray-dried skim milk. When prepared in a 10% solution, it is equivalent to fresh skim milk.

Skim Milk can be used to prepare skim milk agar for detecting proteolytic microorganisms in foods[1], including dairy products.[2] It can also be used to prepare litmus milk, a differential test medium for determining lactose fermentation and for detecting proteolytic enzymes that hydrolyze casein (milk protein) and cause coagulation (clot formation).[3]

User Quality Control

Identity Specifications

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	10% solution, soluble in distilled or deionized water on warming. Solution is white, opalescent. After autoclaving, solution is off-white to beige, opaque.
Reaction of 10% Solution at 25°C:	pH 6.3 ± 0.2
Chemical Test:	Positive reaction with 3,5-dinitro salicylic acid.*

* Place 2 drops of a 2% solution of Skim Milk on filter paper and air dry. Dispense 3 drops of 0.5% 3,5-dinitro salicylic acid in 4% sodium hydroxide over the spot. Heat to 105°C for 5 minutes and note color development. A positive test is indicated by development of a brown color.

Cultural Response

Prepare Skim Milk per label directions. Inoculate with a drop or loopful of undiluted culture and incubate the tubes at 35 ± 2°C for 1-7 days.

ORGANISM	ATCC®	GROWTH	APPEARANCE
Lactobacillus casei	9595	good	acid, reduction, curd
Escherichia coli	25922*	good	acid, reduction, curd
Clostridium perfringens	12919	good	stormy fermentation

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

Skim Milk is a source of lactose and casein. In the differential test medium, Litmus Milk, lactose fermentation is detected by the pH indicator, litmus. Hydrolysis of casein is detected by visible formation of a clot.

Formula

Skim Milk

Formula Per Liter

Skim Milk . . . . 100 g

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.

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

Skim Milk

Materials Required but not Provided

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

Method of Preparation

1. Dissolve 100 grams in 1 liter distilled or deionized water (with warming, if necessary).
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure
See appropriate references for specific procedures.

Results
Refer to appropriate references and procedures for results.

Limitations of the Procedure
Skim Milk supports growth of many microorganisms. Perform microscopic examination and other biochemical tests to identify isolates to the genus and species level, if necessary.

References

1. **Lee, J. S., and A. A. Kraft.** 1992. Proteolytic microorganisms, p. 193-198. *In* C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Frank, J. F., G. L. Christen, and L. B. Bullerman.** 1993. Tests for groups of microorganisms, p. 271-286. *In* Marshall, R. T. (ed.) Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **MacFaddin, J. F**. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 440-445. Williams & Wilkins, Baltimore, MD.

Packaging
Skim Milk	500 g	0032-17

Bacto® Snyder Test Agar

Intended Use
Bacto Snyder Test Agar is used for estimating the relative number of lactobacilli in saliva based on acid production.

Also Known As
BCG Dextrose Agar[1]

Summary and Explanation
Tooth decay (dental caries) is a localized, progressive demineralization of the hard tissues of the crown and root surfaces of teeth. *Streptococcus mutans* and possibly lactobacilli ferment dietary carbohydrates that produce acids that cause the de- mineralization. The organisms reside in dental plaque, which is a gelatinous material that adheres to the surfaces of teeth. Demineralization of the tooth alternates with periods of remineralization. If demineralization exceeds remineralization, a subsurface carious lesion becomes a clinical cavity with extension of the decay into the dentine.[2]

Snyder [3,4] described a test procedure for determining, by colorimetric analysis, the rate and amount of acid produced by microorganisms in saliva. The procedure uses an agar medium that is known as Snyder Test Agar. Alban[5] simplified the procedure, used it extensively and reported it to be more accurate than Snyder's original procedure.

Principles of the Procedure
Snyder Test Agar contains Tryptose as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate. Brom Cresol Green is the pH indicator. Bacto Agar is the solidifying agent.

Microorganisms that use the dextrose in the medium acidify the medium and the pH indicator, brom cresol green, changes color from blue-green to yellow.

User Quality Control

Identity Specifications
Dehydrated Appearance: Light green, free-flowing, homogeneous.

Solution: 6.5% solution, soluble in distilled or deionized water on boiling. Solution is dark emerald green, slightly opalescent.

Prepared Medium: Dark emerald green, slightly opalescent.

Reaction of 6.5% Solution at 25°C: pH 4.8 ± 0.2

Cultural Response
Prepare Snyder Test Agar per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Lactobacillus casei	9595	100-1,000	good	+
Lactobacillus fermentum	9338	100-1,000	good	+

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

Uninoculated tube — *Lactobacillus fermentum* ATCC® 9338

Formula

Snyder Test Agar

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	20 g
Sodium Chloride	5 g
Bacto Agar	20 g
Brom Cresol Green	0.02 g

Final pH 4.8 ± 0. 2 at 25°C

Precautions

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

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

Snyder Test Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)
Waterbath (45°C)
Cotton Swab
Paraffin

Method of Preparation

1. Suspend 65 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Specimens should be collected preferably before breakfast, lunch, or dinner, and before the teeth are brushed. This procedure can be done just before lunch or dinner.

Test Procedure

Snyder Procedure [3,4]

1. Collect specimens of saliva in a sterile container while patient is chewing paraffin for 3 minutes.
2. Shake specimens thoroughly and transfer 0.2 ml to a tube of sterile Snyder Test Agar melted and cooled to 45°C. (Prepared medium in tubes is heated in a boiling water bath for 10 minutes and cooled to 45°C.
3. Rotate the inoculated tubes to mix the inoculum uniformly with the medium and allow to solidify in an upright position.
4. Incubate at 35°C. Observe color at 24, 48 and 72 hours.

Alban Modication

1. Collect enough unstimulated saliva to just cover the medium in the tube. When specimen collection is difficult, dip a sterile cotton swab into the saliva under the tongue or rub on tooth surfaces and place the swab just below the surface of the medium.
2. Incubate the inoculated tubes and an uninoculated control at 35°C.
3. Examine tubes daily for four days.
4. Observe daily color change compared to control tube.

Results

Snyder Procedure

Observe tubes for a change in color of the medium from bluish-green (control) to yellow. A positive reaction is a change in color so that green is no longer dominant. Record as ++ to ++++. A negative reaction is no change in color or only a slight change. Green is still dominant. Record as 0 to +.

Interpretation:

	HOURS INCUBATION		
CARIES ACTIVITY	24	48	72
Marked	Positive	–	–
Moderate	Negative	Positive	–
Slight	Negative	Negative	Positive
Negative	Negative	Negative	Negative

Data summarizing the correlation between the Snyder colorimetric test and *Lactobacillus* counts on specimens of saliva collected routinely are tabulated.

Alban Modification

a. No color change
b. Color beginning to change to yellow from top of medium down (+)
c. One half of medium yellow (++)
d. Three fourths of medium yellow (+++)
e. The entire medium is yellow (++++)

The final report is a composite of the daily readings, for example; – + ++ +++. The readings indicate the rapidity and amount of acid production.

Limitations of the Procedure

1. The data indicate only what is happening at the time the specimen was collected.
2. At least two specimens collected with 2-4 days must be obtained to establish a base-line or reference point.
3. Only when two or more specimens have been cultured can any reliability or prediction be obtained.
4. The clinician must study enough cases by use of periodic laboratory data to establish the value of significance for the purpose intended.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 713-715. vol. 1. Williams & Wilkins, Baltimore, MD.

2. **Lewis, D. W., and A. I. Ismail.** 1995. Periodic health examination, 1995 update: 2. Prevention of dental caries. Canadian Medical Association Journal **152**:836- 846.
3. **Snyder**. 1941. J. Dent. Res. **20**:189.
4. **Snyder**. 1941. J. Am. Dent. Assoc. **28**:44.
5. **Alban**. 1970. J. Dent. Res. **49**:641.

Packaging

Snyder Test Agar	500 g	0247-17

Bacto® Soytone
Bacto Soytone No. 2

User Quality Control

Identity Specifications

Soytone, Soytone No. 2

Dehydrated Appearance: Light to medium tan, free-flowing, homogenous.

Solution: 2% solution, soluble in distilled or deionized water. Light to medium amber, clear to very slightly opalescent.

Reaction of 1% Solution at 25°C: pH 7.0 ± 0.5

Cultural Response

TEST	SOLUTION OF SOYTONE OR SOYTONE NO.2	ORGANISM	ATCC®	SOYTONE RESULT	SOYTONE NO.2 RESULT
Fermentable Carbohydrate	2%	*Escherichia coli*	25922*	positive	negative
Indole Production	0.1%	*Escherichia coli*	25922*	positive	positive
Acetylmethyl-carbinol Production	1% w/0.5% NaCl and 0.5% dextrose	*Enterobacter aerogenes*	13048*	positive	positive
Hydrogen Sulfide Production	1%	*Salmonella typhimurium*	14028*	positive	positive

TEST	SOLUTION OF SOYTONE OR SOYTONE NO.2	ORGANISM	ATCC®	RESULT
Growth Response	2% w/0.5% NaCl and 1.5% agar	*Brucella suis*	4314	good growth
Growth Response	2% w/0.5% NaCl and 1.5% agar	*Escherichia coli*	25922*	good growth
Growth Response 1.5% agar	2% w/0.5% NaCl and	*Staphylococccus aureus*	25923*	good growth

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

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

Intended Use

Bacto Soytone and Bacto Soytone No. 2 are enzymatic digests of soybean meal.

Also Known As

Soytone is also known as Peptone S and Peptone Soya.

Summary and Explanation

Soytone and Soytone No. 2 are enzymatic hydrolysates of soybean meal prepared under controlled conditions for use in microbiological procedures. They are recommended for use in media for the cultivation of a large variety of organisms, including fungi and microbiological assay media. The nitrogen source in Soytone and Soytone No. 2 contains the naturally occurring high concentrations of vitamins and carbohydrates of soybean. Media supplemented with blood produce typical bacterial hemolytic patterns with Soytone and Soytone No. 2 as the main source of nitrogen.

Soytone No. 2 minimizes Bovine Spongiform Encephalopathy (BE) risk in vaccine production because the enzyme used is also of plant origin.

Principles of the Procedure

Soytone is an enzymatic digest of soybean meal.

Soytone No. 2 is a papaic digestion of soybean meal.

Typical Analysis

Soytone

Physical Characteristics

Ash (%)	12.0	Loss on Drying (%)	4.6
Clarity, 1% Solution (NTU)	1.0	pH, 1% Solution	7.2
Filterability (g/cm^2)	1.2		

Carbohydrate (%)

Total	24.0

Nitrogen Content (%)

Total Nitrogen	9.4	AN/TN	33.0
Amino Nitrogen	3.1		

Amino Acids (%)

Alanine	2.46	Lysine	3.45
Arginine	3.82	Methionine	0.86
Aspartic Acid	7.27	Phenylalanine	2.46
Cystine	1.45	Proline	2.92
Glutamic Acid	12.76	Serine	2.87
Glycine	2.51	Threonine	2.17
Histidine	1.24	Tryptophan	0.47
Isoleucine	2.37	Tyrosine	1.93
Leucine	4.03	Valine	2.65

Inorganics (%)

Calcium	0.055	Phosphate	0.820
Chloride	0.165	Potassium	2.220
Cobalt	<0.001	Sodium	3.404
Copper	<0.001	Sulfate	2.334
Iron	0.008	Sulfur	1.660
Lead	<0.001	Tin	<0.001
Magnesium	0.161	Zinc	0.001
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	9.0
Choline (as Choline Chloride)	2200.0	Pantothenic Acid	13.0
Cyanocobalamin	<0.1	Pyridoxine	11.0
Folic Acid	3.0	Riboflavin	<0.1
Inositol	2100.0	Thiamine	1.2
Nicotinic Acid	19.1	Thymidine	113.2

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	38
Salmonella	negative	Thermophile Count	<3
Spore Count	10		

Precautions

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

Storage

Store Soytone and Soytone No. 2 below 30°C. The products are 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

Soytone
Soytone No. 2

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Soytone or Soytone No. 2 in the formula of the medium being prepared. Add Soytone or Soytone No. 2 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 Soytone or Soytone No. 2.

Results

Refer to appropriate references and procedures for results.

Packaging

Soytone	500 g	0436-17
	10 kg	0436-08
Soytone No. 2	500 g	0508-17
	10 kg	0508-08

Bacto® Spirit Blue Agar Lipase Reagent

Intended Use

Bacto Spirit Blue Agar is for use with Bacto Lipase Reagent or other lipid source for detecting and enumerating lipolytic microorganisms.

Summary and Explanation

In 1941, Starr[1] described a lipid emulsion medium for detecting lipolytic (lipase-producing) microorganisms to which he added the dye, spirit blue. Other dyes as indicators of lipolysis were toxic to many microorganisms. Spirit blue did not have toxic effects. When testing samples of dairy products, air and sewage on Spirit Blue Agar, Starr obtained accurate counts of lipolytic microorganisms and total microbial counts on the same medium.

Lipolytic microorganisms, such as psychrotrophic bacteria, molds or yeasts, can adversely affect the flavor of milk and high fat dairy products. Spirit Blue Agar is a recommended medium for testing milk and dairy products.[2]

Lipase Reagent, a mixture of tributyrin and Polysorbate 80, is recommended as the lipid source. Other lipoidal emulsions may be prepared from cottonseed meal, cream, Wesson® oil and olive oil. A satisfactory emulsion can be prepared by dissolving 10 grams gum acacia or 1 ml Tween 80® in 400 ml warm distilled water, adding 100 ml cottonseed or olive oil and agitating vigorously to emulsify.

Principles of the Procedure

Spirit Blue Agar contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Spirit Blue is the indicator of lipolysis. Bacto Agar is the solidifying agent.

Lipase Reagent contains tributyrin, a true fat and the simplest triglyceride occurring in natural fats and oils. It is a good substrate when testing for lipolytic microorganisms because some microorganisms that hydrolyze tributyrin will not hydrolyze other triglycerides or fats containing longer chain fatty acids.[2]

Formula

Spirit Blue Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Agar	20 g
Spirit Blue	0.15 g

Final pH 6.8 ± 0.2 at 25°C

Lipase Reagent

A ready-to-use lipid suspension, containing a mixture of tributyrin and Polysorbate 80.

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 Lipase Reagent 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

Spirit Blue Agar
Lipase Reagent

Materials Required but not Provided

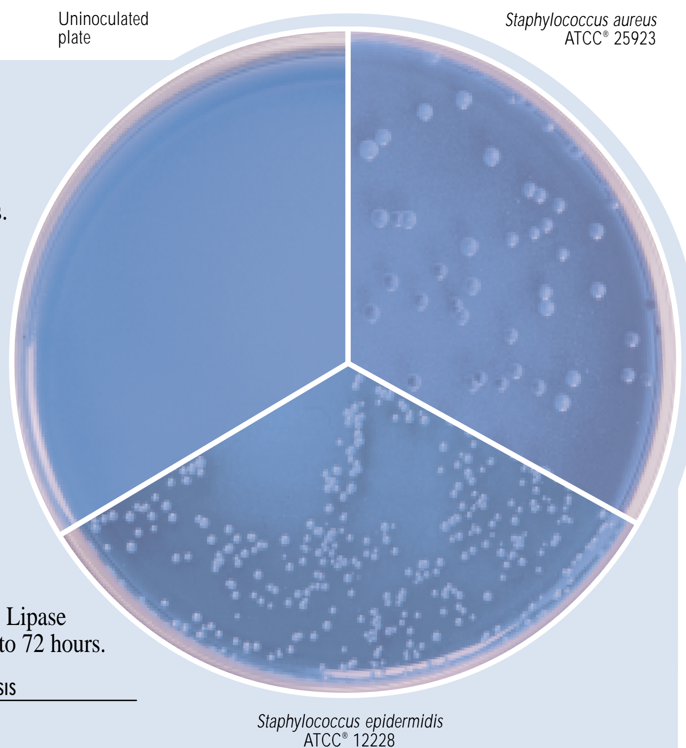
Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 35 grams of Spirit Blue Agar in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Aseptically add 30 ml Lipase Reagent or other lipid source and mix thoroughly.

Specimen Collection and Preparation

Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.



User Quality Control

Identity Specifications

Spirit Blue Agar

Dehydrated Appearance:	Grayish-beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is royal blue, slightly opalescent.
Prepared Medium:	plain - royal blue, opalescent plain + 3% Lipase reagent - pale blue, opalescent
Reaction of 3.5% Solution at 25°C:	pH 6.8 ± 0.2

Lipase Reagent

Appearance:	White, opaque emulsion

Cultural Response

Prepare Spirit Blue Agar per label directions, with the addition of 3% Lipase Reagent after sterilization. Inoculate and incubate at 35 ± 2°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HALO/LIPOLYSIS
Proteus mirabilis	25933	100-1,000	good	no halo
Staphylococcus aureus	25923*	100-1,000	good	halo
Staphylococcus aureus	6538	100-1,000	good	halo
Staphylococcus epidermidis	12228*	100-1,000	good	halo

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

Test Procedure

1. Inoculate organism onto medium.
2. Incubate plates at 35 ± 2°C for up to 72 hours.

Results

Lipolytic microorganisms metabolize the lipid in the medium and form colonies with halos indicating lipolysis.

Limitations of the Procedure

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

References

1. **Starr, M. P**. 1941. Spirit blue agar: a medium for the detection of lipolytic microorganisms. Science **93**:333-334.
2. **Frank. J. F., G. L. Christen, and L. B. Bullerman.** 1993. Tests for groups of microorganisms, p. 276-277. *In* R. T. Marshall (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Spirit Blue Agar	100 g	0950-15
	500 g	0950-17
Lipase Reagent	6 x 20 ml	0431-63

Bacto® m Staphylococcus Broth

Intended Use

Bacto m Staphylococcus Broth is used for isolating staphylococci by the membrane filtration technique.

User Quality Control

Identity Specifications

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

Solution: 10.4% solution, soluble in distilled or deionized water on warming. Solution is light amber, clear to slightly opalescent, may have a slight precipitate.

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

Reaction of 10.4% Solution at 25°C: pH 7.0 ± 0.2

Cultural Response

Prepare m Staphylococcus Broth per label directions. Use the membrane filtration technique with the test organisms. Inoculate and incubate at 35 ± 2°C under humid conditions for 40-48 hours. Plates are read for recovery and pigment production. Mannitol fermentation is detected by adding a drop of Brom Thymol Blue to the site where a colony was removed. Yellow color indicates a positive result for Mannitol fermentation.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	MANNITOL FERMENTATION	PIGMENT PRODUCTION
Escherichia coli	25922*	20-200	inhibited	N/A	–
Staphylococcus aureus	25923*	20-200	good	+	+
Staphylococcus epidermidis	12228*	20-200	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

Staphylococci, along with other bacteria, are indicators of recreational water quality.[4] Indicators of health risk include normal skin flora that are likely to be shed, such as *Pseudomonas*, *Streptococcus*, and *Staphylococcus*.[5] These organisms account for a large percentage of swimming pool-associated illness.[4]

The coagulase-positive species, *Staphylococcus aureus,* is well documented as a human opportunistic pathogen.[3] Coagulase-negative *Staphylococcus* spp. are a major component of the normal microflora of humans.[3] Staphylococci are widespread in nature, though they are mainly found living on the skin, skin glands, and mucous membranes of mammals and birds.[3]

Chapman[1] added 7.5% NaCl to Phenol Red Mannitol Agar to achieve a selective medium for staphylococci. While studying this medium formulation, Chapman[2] developed Staphylococcus Medium 110. m Staphylococcus Broth is patterned after the formula of Staphylococcus Medium 110.

m Staphylococcus Broth, with the addition of sodium azide, is specified for Recreational Waters in Standard Methods for the Examination of Water and Wastewater.[4]

Principles of the Procedure

Tryptone provides the nitrogen, amino acids and minerals in m Staphylococcus Broth. Yeast Extract is the vitamin source in this formula. Lactose and Mannitol are the carbohydrates for bacterial growth. Dipotassium Phosphate is the buffering agent. The high concentration of Sodium Chloride permits this medium to be selective for staphylococci.

Formula

m Staphylococcus Broth

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	2.5 g
Bacto Lactose	2 g
Bacto Mannitol	10 g
Dipotassium Phosphate	5 g
Sodium Chloride	75 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

m Staphylococcus Broth

Materials Required But Not Provided

Membrane filtration equipment
Membrane filter
Autoclave
Glassware
Incubator (35°C)
Sterile tubes
Distilled or deionized water
Paper pads

Method of Preparation

1. Suspend 104 grams in 1 liter distilled or deionized water.
2. Warm to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

NOTE: When autoclave sterilization is not practical, boil medium for 5 minutes.

Specimen Collection and Preparation

Collect water samples as described in Standard Methods, Section 9213[4] or as specified by laboratory procedures.

Test Procedure

1. Follow the membrane filtration procedure described in Standard Methods, Section 9213,[4] or as described by laboratory procedures.
2. Use 2.0-2.5 ml of medium to saturate the paper pads on which the inoculated membrane is placed.
3. Incubate at 35 ± 2°C for 40-48 hours.

Results

Observe tubes for growth, indicating a positive reaction. Inoculate tubes showing turbidity to the appropriate medium for confirmation of *Staphylococcus.*

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. m Staphylococcus Broth is used in sequence with an additional medium for confirmation. If necessary, confirm positive isolates using biochemical reactions.

References

1. **Chapman.** 1945. J. Bacteriol. **50**:201.
2. **Chapman.** 1946. J. Bacteriol. **51**:409.
3. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*, p. 282-298. *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. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Seyfried, P. L., R. S. Tobin, N. E. Brown, and P. F. Ness.** 1985. A prospective study of swimming-related illness. II. Morbidity and the microbiological quality of water. Amer. J. Public Health **75**:1071.

Packaging

m Staphylococcus Broth	100 g	0649-15
	500 g	0649-17

Bacto® Staphylococcus Medium 110

Intended Use

Bacto Staphylococcus Medium 110 is used for isolating and differentiating staphylococci based on mannitol fermentation, pigment formation and gelatinase activity.

Also Known As

Staphylococcus Medium 110 is also known as Staphylococcus Agar No. 110 (Staphy-110, S-110) and Stone Gelatin Agar.[1]

Summary and Explanation

Stone[2] described a culture medium on which food-poisoning staphylococci gave a positive gelatinase test. Chapman, Lieb and Curcio[3] later reported that pathogenic staphylococci strains typically ferment mannitol, form pigment and produce gelatinase. Chapman[4] suggested adding 7.5% NaCl to Phenol Red Mannitol Agar to make a selective isolation medium for staphylococci using a high salt content. Further studies by Chapman[5] led to the development of Staphylococcus Medium 110. This medium is included in standard methods procedures for selectively isolating pathogenic staphylococci from foods.[6]

Principles of the Procedure

Staphylococcus Medium 110 contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Sodium Chloride, in high concentration, inhibits most bacteria other than staphylococci. Lactose and D-Mannitol are the carbohydrates. Gelatin is included for testing liquefaction. Bacto Agar is the solidifying agent.

Pathogenic staphylococci (coagulase-positive staphylococci) typically resist the high salt concentration and form colonies with a yellow-orange pigment. These organisms typically ferment mannitol and produce acid, and liquefy gelatin, producing zones of clearing around the colonies.

Formula

Staphylococcus Medium 110

Formula Per Liter

Bacto Tryptone	10	g
Bacto Yeast Extract	2.5	g
Bacto Gelatin	30	g
Bacto Lactose	2	g
Bacto D-Mannitol	10	g
Sodium Chloride	75	g
Dipotassium Phosphate	5	g
Bacto Agar	15	g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

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

Solution: 14.9% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent to opalescent, with heavy precipitate.

Prepared Medium: Light amber, slightly opalescent to opalescent.

Reaction of 14.9% Solution at 25°C: pH 7.0 ± 0.2

Cultural Response

Prepare Staphylococcus Medium 110 per label directions. Inoculate the plates and incubate the plates at 35 ± 2°C for 18-48 hours.

To test for mannitol fermentation, remove a colony from the medium, add a drop of 0.04% brom thymol blue to the plate, and observe for the formation of a yellow color (positive reaction).

To test for gelatinase reaction, flood the plate with 5 ml of saturated ammonium sulfate solution and incubate at 35 ± 2°C for 10 minutes. Observe for a zone of clearing around the colonies (positive reaction).

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	PIGMENT**	Gelatinase	Mannitol
Escherichia coli	25922*	100-300	marked to complete inhibition	–	N/A	N/A
Staphylococcus aureus	25923*	100-300	good	+	+	+
Staphylococcus epidermidis	12228*	100-300	good	–	+	–

**Pigment is seen as a yellow to orange color.

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

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

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Staphylococcus Medium 110

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)
0.04% Bromthymol blue
Saturated ammonium sulfate solution

Method of Preparation

1. Suspend 149 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Evenly disperse the precipitate when dispensing.

Specimen Collection and Preparation

Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[6]

Test Procedure

Consult appropriate references for procedures concerning selection and enumeration of staphylococci.[6]

Results

Growth of pathogenic staphylococci produces colonies with yellow-orange pigment.

Limitations of the Procedure

1. *Enterococcus faecalis* may grow on Staphylococcus Medium 110 as tiny colonies with mannitol fermentation. Differentiate these organisms from staphylococci with the Gram stain and catalase test.
2. Suspected staphylococci must be subcultured to Nutrient Broth, Blood Agar, BHI Broth, or Tryptose Phosphate Broth for coagulase testing as the high salt content of Staphylococcus Medium 110 may interfere with results.
3. Pigment production is not a reliable criterion for differentiation of staphylococcal spp.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 722-726. Williams & Wilkins, Baltimore, MD.
2. **Stone, R. V.** 1935. A cultural method for classifying staphylococci as of the "food poisoning" type. Proc. Soc. Exptl. Biol. Med. **33**:185-187.
3. **Chapman, G. H., C. W. Lieb, and L. G. Curcio.** 1937. Isolation and cultural differentiation of food-poisoning staphylococci. Food Research. **2**:349.
4. **Chapman, G. H.** 1945. The significance of sodium chloride in studies of staphylococci. J. Bacteriol. **50**:201.
5. **Chapman, G. H.** 1946. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation and the Stone reaction. J. Bacteriol. **51**:409.
6. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Staphylococcus Medium 110	500 g	0297-17
	2 kg	0297-07
	10 kg	0297-08

Bacto® Starch Agar

Intended Use

Bacto Starch Agar is used for cultivating microorganisms being tested for starch hydrolysis.

Summary and Explanation

In 1915,[1] Vedder formulated Starch Agar for cultivating *Neisseria*. Since then, other media have been developed that are superior to Starch Agar for the isolation of *Neisseria* spp, including enriched GC Medium Base. Starch Agar is used in differentiating microorganisms based on the starch hydrolysis test.

Starch Agar Medium for *Pseudomonas*[2] and Starch Agar with Bromcresol Purple[3] are modifications of Starch Agar used for the differentiation of *Gardnerella vaginalis*.

User Quality Control

Identity Specifications

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

Solution: 2.5% solution, soluble in distilled or deionized water on boiling. Light amber, slightly opalescent without precipitate.

Prepared Medium: Light amber, slightly opalescent without significant precipitate.

Reaction of 2.5% Solution at 25°C: pH 7.5 ± 0.2

Cultural Response

Inoculate with a single streak of undiluted test organism and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	RECOVERY	STARCH HYDROLYSIS
Bacillus subtilis	6633	good	positive
Escherichia coli	25922*	good	negative
Staphylococcus aureus	25923*	good	negative
Streptococcus pyogenes	19615*	good	negative

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

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

Principles of the Procedure

Beef Extract provides the nitrogen, vitamins, carbon and amino acids in Starch Agar. Starch reacts with Gram's Iodine to give a blue color. Organisms hydrolyzing starch through amylase production will produce a clearing around the isolate while the remaining medium is blue. Bacto Agar is a solidifying agent.

Formula

Starch Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Soluble Starch	10 g
Bacto Agar	12 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

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

Storage

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

Starch Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Gram Iodine
Sterile Petri dishes

Method of Preparation

1. Dissolve 25 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.
5. Dispense into sterile Petri dishes or as desired.

Specimen Collection and Preparation

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

Test Procedure

Starch Hydrolysis Test

Flood the surface of a 48-hour culture on Starch Agar with Gram Iodine.

For a complete discussion of the collection, isolation and identification of microorganisms, refer to appropriate references.[4,5]

Results

Starch hydrolysis (+) is indicated by a colorless zone surrounding colonies. A blue or purple zone indicates that starch has not been hydrolyzed (-).

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. **Vedder.** 1915. J. Infect. Dis. **16**:385.
2. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 844-845, CRC Press, Boca Raton, FL.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 727-729, Williams & Wilkins, Baltimore, MD.
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. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Starch Agar	500 g	0072-17

Bacto® Stock Culture Agar

Intended Use

Bacto Stock Culture Agar is used for maintaining stock cultures of bacteria, particularly streptococci.

User Quality Control

Identity Specifications

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

Solution: 5.0% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, opalescent.

Prepared Medium: Medium amber, opalescent.

Reaction of 5% Solution at 25°C: pH 7.5 ± 0.2

Cultural Response

Prepare Stock Culture Agar per label directions. Inoculate undiluted broth cultures of the test organisms by stabbing the medium with an inoculating needle. Incubate at 35°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH
Staphylococcus aureus	25923*	good
Streptococcus pneumoniae	6305	good
Streptococcus pyogenes	19615*	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

Ayers and Johnson[1] reported a medium that gave luxuriant growth and extended viability of streptococci and other organisms. The success of their medium can be attributed to its semisolid consistency, added casein, buffered environment and dextrose, which serves as a readily available source of energy. This study reported that pathogenic streptococci remained viable for at least four months at room temperature (24°C) in the medium. Organisms such as *Streptococcus pneumoniae*, *Mycobacterium* spp. and others, grew well on their medium. Stock Culture Agar is prepared to duplicate the medium described by Ayers and Johnson.[1]

Stock Culture Agar may also be prepared with L-asparagine (1 gram/liter) for the maintenance of pathogenic and non-pathogenic bacteria, especially streptococci.[2]

Principles of the Procedure

Infusion from Beef Heart, Proteose Peptone, Gelatin and Isoelectric Casein provide the nitrogen, vitamins and amino acids in Stock Culture Agar. Dextrose is a carbon source. Disodium phosphate is a buffering agent. Sodium citrate acts as a preservative. Bacto Agar is a solidifying agent.

Formula

Stock Culture Agar

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Proteose Peptone	10 g
Bacto Gelatin	10 g
Isoelectric Casein	5 g
Bacto Dextrose	0.5 g
Disodium Phosphate	4 g
Sodium Citrate	3 g
Bacto Agar	7.5 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

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

Storage

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

Expiration Date

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

Procedure

Materials Provided

Stock Culture Agar

Materials Required But Not Provided

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

Method of Preparation

1. Suspend 50 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. Dispense as desired.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

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

References

1. **Ayers, S. H., and W. T. Johnson.** 1924. Studies of the streptococci. J. Bacteriol. **9**:111-114.
2. **Atlas, R. M.** 1993. Handbook of microbiological media. CRC Press, Boca Raton, FL.

Packaging

Stock Culture Agar	500 g	0054-17

Bacto® Sulfite Agar

Intended Use

Bacto Sulfite Agar is used for detecting thermophilic, H_2S-producing anaerobes, particularly in foods.

Summary and Explanation

Sulfide spoilage of foods is due to three factors: high spore counts, the heat resistance of the spores, and subjecting the finished product to elevated temperatures. The last factor may occur if the processed food is not cooled adequately.[3]

Clark and Tanner[1] described the thermophilic organisms that cause spoilage in canned foods as flat-sour spoilage organisms, thermophilic anaerobes and sulfide-spoilage organisms. They used Sulfite Agar to study sulfide-spoilage organisms in sugar and starch.

Both beet and cane sugar can carry spores of the thermophilic bacteria that are spoilage agents.[2] *Desulfotomaculum nigrificans*, first classified as *Clostridium nigrificans,* causes spoilage in non-acid canned foods such as vegetables and infant formula.[3] The growth of *D. nigrificans* occurs in the range of pH 6.2-7.8, with the best growth occurring at pH 6.8-7.3. Scanty growth can be observed at pH 5.6. The reaction of most vegetables, except corn and peas, falls below pH 5.8, so sulfide spoilage is rare.[3]

Sulfite Agar is a recommended Standard Methods medium for isolating *D. nigrificans*.[2, 3]

Principles of the Procedure

Sulfite Agar contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Sodium Sulfite, upon reduction, produces hydrogen sulfide. Bacto Agar is the solidifying agent.

Iron nails or iron strips will combine with any dissolved oxygen in the medium and provide an anaerobic environment.

Formula

Sulfite Agar

Formula Per Liter

Bacto Tryptone	10 g
Sodium Sulfite	1 g
Bacto Agar	20 g

Final pH 7.6 ± 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
Sulfite Agar

Materials Required but not Provided
Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Sterile tubes with closures
Iron nails or strips

Method of Preparation
1. Suspend 31 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation[3]

Dry Sugar
1. Place 20 grams of dry sugar in a dry, sterile, graduated 250 ml Erlenmeyer flask closed with a rubber stopper.
2. Add sterile water to the 100 ml mark and shake to dissolve.
3. Replace the stopper with a sterile cotton plug, bring the solution rapidly to a boil, and continue boiling for 5 minutes.
4. Replace evaporated liquid with sterile water.
5. Cool immediately in cold water.

Liquid Sugar
Prepare as for dry sugar except determine the amount of liquid sugar needed on the basis of %Brix in order to be equivalent to 20 grams of dry sugar.[2]

Starch and Flour
1. Place 20 grams of starch or flour in a dry, sterile, graduated 250 ml Erlenmeyer flask.
2. Add sterile water to the 100 ml mark, swirling occasionally.
3. Close the flask with a sterile rubber stopper.
4. Shake well to obtain a uniform, lump-free suspension. Add sterile glass beads to the sample mixture to aid in thoroughly mixing during shaking.

Nonfat Dry Milk
1. Place 10 grams of nonfat dry milk in a sterile, graduated 250 ml Erlenmeyer flask.
2. Add .02N sodium hydroxide to the 100 ml mark.
3. Shake to completely dissolve.
4. Autoclave at 5 pounds pressure for 10 minutes.
5. Cool immediately.

Cream
1. Mix 2 grams of gum tragacanth and 1 gram of gum arabic in 100 ml of water in an Erlenmeyer flask.
2. Sterilize at 121°C for 20 minutes.
3. Transfer 20 ml of cream sample to a sterile, graduated 250 ml Erlenmeyer flask.
4. Add sterilized gum mixture to the 100 ml mark.
5. Shake carefully using a sterile rubber stopper.
6. Loosen the stopper. Autoclave at 5 pounds pressure for 5 minutes.

Soy Protein Isolates
1. Prepare a 10% suspension of soy protein isolate in sterile 0.1% peptone water in milk dilution or similar bottles.

User Quality Control

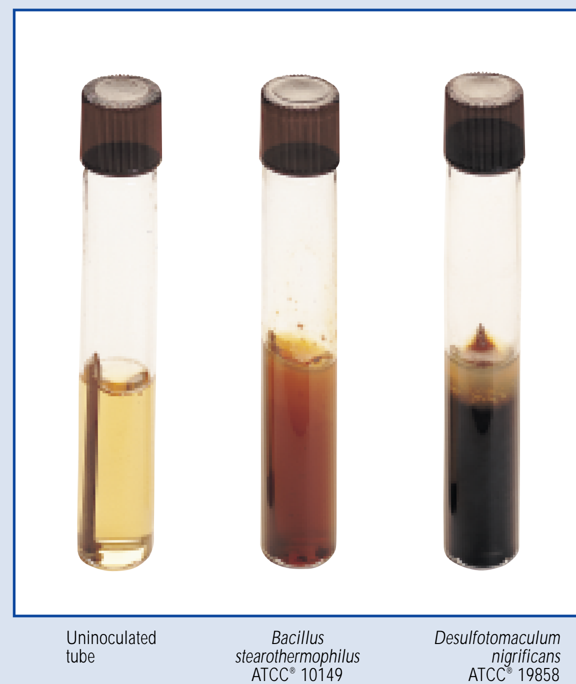
Identity Specifications

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in distilled or deionized water upon boiling. Light amber, very slightly to slightly opalescent.
Prepared Medium:	Light amber, very slightly to slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response
Prepare Sulfite Agar per label directions. Inoculate molten medium, solidify, and incubate aerobically at 55 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	SULFITE REDUCTION
Bacillus stearothermophilus	10149	30-100	good	–
Clostridium thermosaccharolyticum	7956	30-100	good	+
Desulfotomaculum nigrificans	19858	30-100	good	+

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



Uninoculated tube — *Bacillus stearothermophilus* ATCC® 10149 — *Desulfotomaculum nigrificans* ATCC® 19858

2. Adjust to pH 7.0 ± 0.1.
3. Autoclave at 5 pounds pressure for 20 minutes.

Test Procedure

Sugar

1. Divide 20 ml of heated sugar solution among 6 screw-cap tubes (20 x 150 mm) containing approximately 10 ml of freshly autoclaved, still molten Sulfite Agar and a nail.
2. Cool and solidify immediately in cold water.
3. Preheat the tubes to 50-55°C.
4. Incubate at 50-55°C for 24-48 hours.

Starch and Flour

1. Divide 20 ml of the starch or flour suspension among 6 screw-cap tubes (20 x 150 mm) containing approximately 10 ml of freshly autoclaved, still molten Sulfite Agar and a nail.
2. Swirl the tubes several times to ensure even dispersion of the starch or flour in the medium. Heat in a boiling water bath for 15 minutes, continuing to swirl the tubes.
3. Cool and solidify immediately in cold water.
4. Preheat the tubes to 50-55°C.
5. Incubate at 50-55°C for 24-48 hours.

Nonfat Dry Milk

1. Transfer 2 ml of nonfat dry milk solution to each of 2 screw cap tubes (20 X 150 mm) containing freshly autoclaved, still molten Sulfite Agar and a nail.
2. Gently swirl several times.
3. Cool and solidify immediately in cold water.
4. Preheat the tubes to 50-55°C.
5. Incubate at 50-55°C for 24-48 ± 3 hours.
6. Count colonies of *D. nigrificans* and report on the basis of a 10 gram sample.

Soy Protein Isolates

1. Add 1 ml of soy protein isolate suspension to each of 10 tubes containing freshly autoclaved, still molten Sulfite Agar and a nail. If using already prepared medium, heat the tubes immediately before inoculation to eliminate oxygen.
2. Mix tubes.
3. Solidify in an ice water bath.
4. Overlay with Vaspar.
5. Preheat the tubes to 55°C.
6. Incubate at 55°C for 14 days. Take preliminary counts at 48 hours, 7 days and 14 days in case tubes become completely blackened.
7. Count the blackened areas for each tube and report as the number of spores per gram of soy isolate.

Results

Hydrogen sulfide production from the reduction of sulfite causes a blackening of the medium.

Sulfide spoilage spores should be present in not more than 2 of 5 samples tested (40%) with not more than 5 spores per 10 gram in any one sample.[4]

Limitations of the Procedure

1. Nails or iron strips should be cleaned in hydrochloric acid and rinsed well to remove any rust before being placed into tubes of medium.
2. If iron nails or iron strips are not available, substitute 10 ml of 5% ferric citrate solution.
3. Spoiled peas may not show discoloration but will show blackening with a dark- colored brine.
4. Spangling of the enamel may occur as a result of the interaction of dissolved hydrogen sulfide with the iron of the container.

References

1. **Clark, F. M., and F. W. Tanner.** 1937. Thermophilic canned-food spoilage organisms in sugar and starch. Food Res. **2**:27-39.
2. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
3. **Donnelly, L. S., and R. R. Graves.** 1992. Sulfide spoilage sporeformers, p. 317- 323. *In* C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **NCA Research Laboratories.** 1968. Laboratory Manual for Food Canners and Processors, vol. 1, p. 104. Natl. Canners Assn. (Now, Natl. Food Processors Assn.) AVI Inc., Westport, CT.

Packaging

Sulfite Agar	500 g	0972-17

Bacto® Synthetic Broth AOAC

Intended Use

Bacto Synthetic Broth AOAC is used for maintaining disinfectant test cultures.

Summary and Explanation

Synthetic Broth AOAC is a chemically defined broth recommended by the Association of Official Analytical Chemists (AOAC).[1] It contains all the nutrients essential for growth of the test cultures used in determining the phenol coefficients of disinfectants.

Principles Of The Procedure

The chemically-defined ingredients in Synthetic Broth AOAC provide nitrogen, carbon, vitamins and minerals required for bacterial growth.

Formula

Synthetic Broth AOAC

Formula Per Liter

L-Cystine	0.05 g
DL-Methionine	0.37 g
L-Arginine HCl	0.4 g

DL-Histidine HCl	0.3	g
L-Lysine HCl	0.85	g
L-Tyrosine	0.21	g
DL-Threonine	0.5	g
DL-Valine	1	g
L-Leucine	0.8	g
DL-Isoleucine	0.44	g
Glycine	0.06	g
DL-Serine	0.6	g
DL-Alanine	0.43	g
L-Glutamic Acid HCl	1.3	g
L-Aspartic Acid	0.45	g
DL-Phenylalanine	0.26	g
DL-Tryptophan	0.05	g
L-Proline	0.05	g
Sodium Chloride	3	g
Potassium Chloride	0.2	g
Magnesium Sulfate Anhydrous Reagent	0.05	g
Potassium Phosphate	1.5	g
Disodium Phosphate	4	g
Thiamine HCl	0.01	g
Nicotinamide	0.01	g

Final pH 7.1 ± 0.1 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.

User Quality Control

Identity Specifications

Dehydrated Appearance: White, homogeneous, free-flowing.

Solution: 1.7% solution, soluble in distilled or deionized water on boiling. Solution is colorless and clear with no precipitate.

Prepared Medium: Colorless and clear with no precipitate.

Reaction of 1.7% Solution at 25°C: pH 7.1 ± 0.1

Cultural Response

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

ORGANISM	ATCC®	APPROXIMATE INOCULUM CFU	GROWTH
Pseudomonas aeruginosa	15442	100	good
Salmonella choleraesuis	10708	100	good
Salmonella typhi	6539	100	good
Staphylococcus aureus	6538	100	good

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

Storage

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

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

Synthetic Broth AOAC

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°)
20 x 150 mm tubes with closures
Sterile 10% dextrose solution

Method of Preparation

1. Suspend 17 grams in 1 liter distilled or deionized water.
2. Boil for 1-2 minutes.
3. Dispense 10 ml amounts into 20 x 150 mm culture tubes.
4. Autoclave at 121°C for 20 minutes.
5. Before inoculating, aseptically add 0.1 ml sterile 10% dextrose solution to each tube.

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

Not applicable

References

1. **Association of Official Analytical Chemists**. 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Synthetic Broth AOAC	500 g	0352-17
	10 kg	0352-08