PALCAM Medium

Bacto® PALCAM Medium Base • Bacto PALCAM Antimicrobial Supplement

**Intended Use**

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

**Summary and Explanation**

PALCAM Medium Base and PALCAM Antimicrobial Supplement are based on the PALCAM agar formulation of van Netten et al., 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, and by the IDF as an additional plating medium for the detection of *Listeria* spp. in milk and milk products. PALCAM medium is recommended by Health Canada for the detection of *L. monocytogenes* in food and environmental samples.

**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 Antimicrobial 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 dihydroxyxoumarin, 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 Antimicrobial 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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM</th>
<th>GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>inhibited</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>19114</td>
<td>100-1,000</td>
<td>good growth, gray-green colonies with black precipitate</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923*</td>
<td>1,000-2,000</td>
<td>inhibited</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>29212*</td>
<td>1,000-2,000</td>
<td>inhibited</td>
</tr>
</tbody>
</table>

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 Antimicrobial 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 Antimicrobial Supplement:

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

   **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 Antimicrobial 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 Antimicrobial 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 Antimicrobial Supplement

Modified Oxford Antimicrobial Supplement

Listeria Enrichment Broth

Modified Listeria Enrichment Broth

Tryptic Soy Agar with 0.6% Yeast Extract

LPM Agar Base

Moxalactam Antimicrobial 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 Antimicrobial 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. 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**

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**

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**

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. Colonies of mannitol-fermenting organisms such as staphylococci, which may grow on this medium, appear yellow with a yellow halo.

**References**


**Packaging**

<table>
<thead>
<tr>
<th>PALCAM Medium Base</th>
<th>500 g</th>
<th>0636-17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 kg</td>
<td>0636-07</td>
</tr>
</tbody>
</table>

| PALCAM Antimicrobial 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 β-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 reported the presence of a urine phenylalanine metabolite in mentally retarded persons. Jervis 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. Bacillus subtilis ATCC® 6633 growth is inhibited in minimal culture medium containing β-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.3,5

**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

**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
- Magnesium Chloride ..................................... 0.05 g
- Ferric Chloride .......................................... 0.005 g
- Calcium Chloride ........................................ 0.005 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: 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

**Bacillus 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 Thiénylalanine
Subtilis Spore Suspension No. 2

Materials Required but not Provided
β-2-thiénylalanine (use with PKU Test Agar w/o Thiénylalanine)
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 Thiénylalanine, only: Add 1 ml of 0.33% β-2-thiénylalanine 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.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 Thiénylalanine 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.

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
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 Barile9 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 0473-25 50 g
Bacto Peptone 0473-29 10 g
Sodium Chloride 0474-12 5 g
Bacto Agar 0474-18 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 0473-25 50 g
Bacto Peptone 0473-29 10 g
Sodium Chloride 0474-12 5 g
Final pH 7.8 ± 0.2 at 25°C

Mycoplasma Supplement
Ingredients per 30 ml vial
Bacto Yeast Extract 0474-17 0.01 g
Horse Serum, Desiccated 0474-19 1.6 g

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
**Mycoplasma Supplement S**

Ingredients per 30 ml vial

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Yeast Extract</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Horse Serum, Desiccated</td>
<td>1.6 g</td>
</tr>
<tr>
<td>Penicillin</td>
<td>55,000 units</td>
</tr>
<tr>
<td>Thallium acetate</td>
<td>50 mg</td>
</tr>
</tbody>
</table>

**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**
- **Mycoplasma Supplement**
- **Mycoplasma Supplement S**

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

**Mycoplasma Supplement**

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**

1. **PPLO Agar**

   *PPLO Broth w/o CV* Dissolve 21 grams in 700 ml distilled or deionized water and boil to dissolve completely.

**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% CO₂ for up to 7 days. Subculture to PPLO Agar and incubate at 35 ± 2°C under 5-10% CO₂ for up to 7 days. Examine microscopically for growth on a daily basis.

**INOCULUM ATCC® CFU GROWTH**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM</th>
<th>CFU</th>
<th>GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma bovis</td>
<td>25523</td>
<td>100-1,000</td>
<td>good</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma gallinarum</td>
<td>19708</td>
<td>100-1,000</td>
<td>good</td>
<td></td>
</tr>
</tbody>
</table>

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

N. Pagano, H. Levin, and J. Trejo1 are credited with the original development of the medium. The medium was later modified by Leland, D. S., M. A. Lapworth, R. B. Jones, and M. L. V. French. The modified medium is referred to as the Pagano Levin Agar (PLA).

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

Summary and Explanation
Pagano Levin Base as described by Pagano, Levin, and Trejo1 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 Williamson3 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

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Peptone 10 g</td>
</tr>
<tr>
<td>Bacto Yeast Extract 1 g</td>
</tr>
<tr>
<td>Bacto Dextrose 40 g</td>
</tr>
<tr>
<td>Bacto Agar 15 g</td>
</tr>
<tr>
<td>Final pH 6.0 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC*</th>
<th>Inoculum CFU</th>
<th>Growth</th>
<th>Colony Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>26790</td>
<td>100-1,000</td>
<td>good</td>
<td>cream to light pink</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>white, spreading</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>6121</td>
<td>100-1,000</td>
<td>good</td>
<td>red, light red</td>
</tr>
<tr>
<td>Candida stellatoidea</td>
<td>36232</td>
<td>100-1,000</td>
<td>good</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25292</td>
<td>1,000-2,000</td>
<td>inhibited</td>
<td></td>
</tr>
</tbody>
</table>

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

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 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.

**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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Dextrose</td>
<td>15</td>
</tr>
<tr>
<td>Bacto Vitamin Assay Casamino Acids</td>
<td>2</td>
</tr>
<tr>
<td>Acid Digest of Casein</td>
<td>10</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>0.68</td>
</tr>
<tr>
<td>L-Tryptophane</td>
<td>2</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.16</td>
</tr>
<tr>
<td>Adenine Sulfate</td>
<td>10</td>
</tr>
<tr>
<td>Guanine Hydrochloride</td>
<td>10</td>
</tr>
<tr>
<td>Uracil</td>
<td>10</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>2</td>
</tr>
<tr>
<td>Liver Digest</td>
<td>0.35</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>2</td>
</tr>
<tr>
<td>p- Aminobenzoic Acid</td>
<td>2</td>
</tr>
<tr>
<td>Thiamine Hydrochloride</td>
<td>2</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>2</td>
</tr>
<tr>
<td>Pyridoxine Hydrochloride</td>
<td>2</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>20</td>
</tr>
<tr>
<td>Biotin</td>
<td>16</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>0.8</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>40</td>
</tr>
<tr>
<td>Ferrous Sulfate</td>
<td>40</td>
</tr>
<tr>
<td>Manganese Sulfate</td>
<td>0.16</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>2</td>
</tr>
<tr>
<td>Final pH 6.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

**Panthenol Supplement**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Glycerol</td>
<td>15</td>
</tr>
<tr>
<td>Sorbitan Monooleate Complex</td>
<td>33</td>
</tr>
<tr>
<td>Acid Digest of Casein</td>
<td>2</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>0.15</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.15</td>
</tr>
<tr>
<td>Adenine Sulfate</td>
<td>10</td>
</tr>
<tr>
<td>Guanine Hydrochloride</td>
<td>10</td>
</tr>
<tr>
<td>Uracil</td>
<td>10</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>2</td>
</tr>
<tr>
<td>Liver Digest</td>
<td>0.35</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>2</td>
</tr>
<tr>
<td>p- Aminobenzoic Acid</td>
<td>2</td>
</tr>
<tr>
<td>Thiamine Hydrochloride</td>
<td>2</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>2</td>
</tr>
<tr>
<td>Pyridoxine Hydrochloride</td>
<td>2</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>20</td>
</tr>
<tr>
<td>Biotin</td>
<td>16</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>0.8</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>40</td>
</tr>
<tr>
<td>Ferrous Sulfate</td>
<td>40</td>
</tr>
<tr>
<td>Manganese Sulfate</td>
<td>0.16</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>2</td>
</tr>
<tr>
<td>Final pH 6.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

**User Quality Control**

**Identity Specifications**

<table>
<thead>
<tr>
<th>Panthenol Assay Medium</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated Appearance:</td>
<td>Light beige, homogeneous, free-flowing.</td>
</tr>
<tr>
<td>Solution:</td>
<td>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.</td>
</tr>
<tr>
<td>Prepared Medium (Single-strength):</td>
<td>Very light amber, clear, may have a very slight precipitate.</td>
</tr>
<tr>
<td>Reaction of 1.65% Solution at 25°C:</td>
<td>pH 6.0 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Panthenol Supplement</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution Appearance:</td>
<td>Colorless to very, very light amber, clear.</td>
</tr>
<tr>
<td>Reaction of Solution at 25°C:</td>
<td>pH 5.0-6.0</td>
</tr>
</tbody>
</table>

**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.
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, 1.0, 1.5, 2.0, 2.5, 3.0 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

Packaging
Panthenol Assay Medium
Panthenol Supplement

**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 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.

**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) solution 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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Vitamin Assay Casamino Acids</td>
</tr>
<tr>
<td>Bacto Dextrose</td>
</tr>
<tr>
<td>Sodium Acetate</td>
</tr>
<tr>
<td>L-Cystine</td>
</tr>
<tr>
<td>DL-Tryptophane</td>
</tr>
<tr>
<td>Adenine Sulfate</td>
</tr>
<tr>
<td>Guanine Hydrochloride</td>
</tr>
<tr>
<td>Uracil</td>
</tr>
<tr>
<td>Thiamine Hydrochloride</td>
</tr>
<tr>
<td>Riboflavin</td>
</tr>
<tr>
<td>Niacin</td>
</tr>
<tr>
<td>Pyridoxine</td>
</tr>
<tr>
<td>p-Aminobenzoic Acid</td>
</tr>
<tr>
<td>Biotin</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
</tr>
<tr>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Ferrous Sulfate</td>
</tr>
<tr>
<td>Manganese Sulfate</td>
</tr>
<tr>
<td>Final pH 6.7 ± 0.1 at 25°C</td>
</tr>
</tbody>
</table>

**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 values. 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

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® and Pantothenate Acid Assay in AOAC.® *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.

**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 ........................................ 0.4 g
- Magnesium Sulfate ................................ 0.4 g
- Sodium Chloride ...................................... 20 mg
- Ferrous Sulfate ..................................... 20 mg
- Manganese 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
- Sorbitol Monooleate Complex .................... 0.1 g

**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.

**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
- 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 or AOAC.
1. Prepare stock cultures of *Lactobacillus 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% NaCl. After the third wash, resuspend the cells with sterile 0.85% NaCl 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 or AOAC. 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). Titrimetric 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 and AOAC.

**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**

**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.

**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) 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 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.

**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.

**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.

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

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

Bacto 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. 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. In a study by Morton, Smith and Leberman, 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. Peptone is routinely recommended for culture media preparation. Several media containing Peptone are specified in standard methods for multiple applications.

Pepette 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 |
| Clarity, 1% Solution (NTU) | 0.5 |
| Filterability (g/cm²) | 0.5 |

<table>
<thead>
<tr>
<th>Carbohydrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nitrogen Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nitrogen</td>
</tr>
<tr>
<td>Amino Nitrogen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amino Acids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Aspartic Acid</td>
</tr>
<tr>
<td>Cystine</td>
</tr>
<tr>
<td>Glutamic Acid</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inorganics (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Chloride</td>
</tr>
<tr>
<td>Cobalt</td>
</tr>
<tr>
<td>Copper</td>
</tr>
<tr>
<td>Lead</td>
</tr>
<tr>
<td>Magnesium</td>
</tr>
<tr>
<td>Manganese</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamins (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
</tr>
<tr>
<td>Choline Chloride</td>
</tr>
<tr>
<td>Cyanocobalamin</td>
</tr>
<tr>
<td>Folic Acid</td>
</tr>
<tr>
<td>Inositol</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biological Testing (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform negative</td>
</tr>
<tr>
<td>Salmonella negative</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>TEST</th>
<th>SOLUTION</th>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentable</td>
<td>2%</td>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole</td>
<td>0.1%</td>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>Production</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetymethyl-</td>
<td>0.1%</td>
<td><em>Enterobacter aerogenes</em></td>
<td>13048*</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>carbinol</td>
<td>with 0.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogen</td>
<td>1%</td>
<td><em>Salmonella typhi</em></td>
<td>6539</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>Sulfide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth Response</td>
<td>2% with</td>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>100-1,000</td>
<td>good growth</td>
</tr>
<tr>
<td></td>
<td>1.5% Agar and 0.5% NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth Response</td>
<td>2% with</td>
<td><em>Staphylococcus aureus</em></td>
<td>25923*</td>
<td>100-1,000</td>
<td>good growth</td>
</tr>
<tr>
<td></td>
<td>1.5% Agar and 0.5% NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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. Incubate at 35 ± 2°C for 18-48 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM</th>
<th>CFU</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>100-1,000</td>
<td>good growth</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923*</td>
<td>100-1,000</td>
<td>good growth</td>
<td></td>
</tr>
</tbody>
</table>

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**
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.

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
<th>H₂S PRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>undiluted</td>
<td>good</td>
<td>–</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>13076</td>
<td>undiluted</td>
<td>good</td>
<td>+ (black)</td>
</tr>
</tbody>
</table>

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.

**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

**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 H₂S production. Sodium Glycerophosphate is a buffering compound. Bacto Agar is a solidifying agent.

**Formula**

**Peptone Iron Agar**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Peptone</td>
</tr>
<tr>
<td>Bacto Proteose Peptone</td>
</tr>
<tr>
<td>Ferric Ammonium Citrate</td>
</tr>
<tr>
<td>Sodium Glycerophosphate</td>
</tr>
<tr>
<td>Sodium Thiosulfate</td>
</tr>
<tr>
<td>Bacto Agar</td>
</tr>
</tbody>
</table>

Final pH 6.7 ± 0.2 at 25°C

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

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. 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.
2. For specific information about specimen preparation and inoculation for isolation of coliform bacteria, consult appropriate references.

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.

References


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. This non-selective medium has been used as a basal medium for biochemical tests such as carbohydrate fermentation patterns and production of indole.

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

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone ..................</td>
</tr>
<tr>
<td>Sodium Chloride ........</td>
</tr>
<tr>
<td>Final pH 7.2 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
<th>INDOLE REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>undiluted</td>
<td>good</td>
<td>positive</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
<th>ACID PRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>100-1,000</td>
<td>good</td>
<td>positive</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923*</td>
<td>100-1,000</td>
<td>good</td>
<td>positive</td>
</tr>
</tbody>
</table>

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.

References

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. 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. 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

**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.

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Proteose Peptone No. 3</td>
<td>10</td>
</tr>
<tr>
<td>Bacto Beef Extract</td>
<td>1</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>15</td>
</tr>
<tr>
<td>Bacto Phenol Red</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Lactose Agar

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Proteose Peptone No. 3</td>
<td>10</td>
</tr>
<tr>
<td>Bacto Beef Extract</td>
<td>1</td>
</tr>
<tr>
<td>Lactose</td>
<td>10</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>15</td>
</tr>
<tr>
<td>Bacto Phenol Red</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Mannitol Agar

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Proteose Peptone No. 3</td>
<td>10</td>
</tr>
<tr>
<td>Bacto Beef Extract</td>
<td>1</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>10</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>15</td>
</tr>
<tr>
<td>Bacto Phenol Red</td>
<td>0.025</td>
</tr>
</tbody>
</table>

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


Packaging

<table>
<thead>
<tr>
<th>Packaging</th>
<th>Phenol Red Agar Base</th>
<th>500 g</th>
<th>0098-17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenol Red Lactose Agar</td>
<td>500 g</td>
<td>0100-17</td>
</tr>
<tr>
<td></td>
<td>Phenol Red Mannitol Agar</td>
<td>500 g</td>
<td>0103-17</td>
</tr>
</tbody>
</table>

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. 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 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. 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.

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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Beef Extract</td>
</tr>
<tr>
<td>Bacto Proteose Peptone No. 3</td>
</tr>
<tr>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Bacto Phenol Red</td>
</tr>
</tbody>
</table>

Final pH 7.4 ± 0.2 at 25°C
Section II Phenol Red Carbohydrate Media

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

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
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

**Cultural Response**

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

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>GROWTH</th>
<th>BASE</th>
<th>DEXTROSE</th>
<th>LACTOSE</th>
<th>MANNOSE</th>
<th>SACCHAROSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcaligenes faecalis</td>
<td>8750</td>
<td>good</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>good</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13883*</td>
<td>good</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>12022*</td>
<td>good</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>A = Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G = Gas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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


Bacto® Phenylalanine Agar

Intended Use

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

Summary and Explanation

Butiaux, Osteux, Fresnoy and Moriamez developed a method to differentiate members of the Proteus and Providencia groups from other Enterobacteriaceae based on deamination of phenylalanine.

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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
<th>REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter aerogenes</td>
<td>13048*</td>
<td>100-1,000</td>
<td>good</td>
<td>–</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>13315*</td>
<td>100-1,000</td>
<td>good</td>
<td>+</td>
</tr>
<tr>
<td>Providencia alcalifaciens</td>
<td>9886</td>
<td>100-1,000</td>
<td>good</td>
<td>+</td>
</tr>
</tbody>
</table>

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

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

Also Known As

Phenylalanine Agar is also known as Phenylalanine Deaminase Medium.
other Enterobacteriaceae based on the ability of Proteus and Providencia to deaminate phenylalanine to phenylpyruvic acid by enzymatic activity. Bynae modified this method by incorporating phenylalanine in the medium used to grow the organisms. Ewing, Davis and Reavis 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.

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. 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 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.

References

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**

<table>
<thead>
<tr>
<th>Component</th>
<th>Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptose</td>
<td>10 g</td>
</tr>
<tr>
<td>Bacto Beef Extract</td>
<td>3 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>Phenylethanol</td>
<td>2.5 g</td>
</tr>
</tbody>
</table>

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. Incubate and incubate at 35 ± 2°C under 5-10% CO₂ for 18-48 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>HEMOLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>12453</td>
<td>1,000-2,000</td>
<td>partial</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923</td>
<td>100-1,000</td>
<td>growth</td>
<td>beta</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>6305</td>
<td>100-1,000</td>
<td>growth</td>
<td>alpha</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>19615</td>
<td>100-1,000</td>
<td>growth</td>
<td>beta</td>
</tr>
</tbody>
</table>

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% CO2 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

Packaging
Phenylethanol Agar

<table>
<thead>
<tr>
<th>Size</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 g</td>
<td>0504-15</td>
</tr>
<tr>
<td>500 g</td>
<td>0504-17</td>
</tr>
<tr>
<td>2 kg</td>
<td>0504-07</td>
</tr>
</tbody>
</table>

---

**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 dextran1 and fibrinogen4 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. Phytohaemagglutinin has been used to obtain the plasma suspension of trypanosomes from the blood of infected rats.5

Mitogenic Activity
Nowell6 discovered that phytohaemagglutinin M initiates mitosis in cultures of lymphocytes isolated from peripheral blood. Later, phytohaemagglutinin P was also shown to possess this property. The application of this technique is important in the characterization of chromosomes. A procedure using phytohaemagglutinin-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 phytohaemagglutinin 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 Hughes10 and Hsu and Pomerat.11 The flame drying of slides by Scherz12 and the staining procedure by Rothfels and Siminovitch13 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 Phytohaemagglutinin 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
Phytohaemagglutinin M is a stable, nontoxic, desiccated mucophytohaemagglutinin.

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

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 Phytohaemagglutinin M and Phytohaemagglutinin 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
Phytohaemagglutinin M
Phytohaemagglutinin 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
Phytohaemagglutinin M or the sterile Phytohaemagglutinin 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.

User Quality Control

Identity Specifications
Phytohaemagglutinin 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 Phytohaemagglutinin M or 0.01 ml Phytohaemagglutinin 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.
**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 10^8 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 a 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 pink. If the indicator becomes amber, loosen the cap for an hour or so to allow the escape of CO₂. 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. 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 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.

**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


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

Summary And Explanation

Plate Count Agar was developed by Buchbinder, Baris and Goldstein 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. 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. This formula is specified in Standard Methods for the Examination of Water and Wastewater.
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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone</td>
<td>5 g</td>
</tr>
<tr>
<td>Bacto Yeast Extract</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Bacto Dextrose (Glucose)</td>
<td>1 g</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>15 g</td>
</tr>
</tbody>
</table>

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.

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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC</th>
<th>Inoculum</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus acidophilus</td>
<td>11506</td>
<td>30-300</td>
<td>good</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923</td>
<td>30-300</td>
<td>good</td>
</tr>
</tbody>
</table>

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.
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

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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM</th>
<th>CPU</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>20-80</td>
<td>good to excellent</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923*</td>
<td>20-80</td>
<td>good to excellent</td>
<td></td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Yeast Extract</td>
</tr>
<tr>
<td>Bacto Tryptone</td>
</tr>
<tr>
<td>Bacto Dextrose</td>
</tr>
<tr>
<td>Final pH 7.0 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

**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**


**Packaging**

<table>
<thead>
<tr>
<th>m Plate Count Broth</th>
<th>100 g</th>
<th>0751-15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 g</td>
<td>0751-17</td>
</tr>
</tbody>
</table>

---

**Bacto® Potato Dextrose Agar & 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 products1, 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**

<table>
<thead>
<tr>
<th>Formula per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potatoes, Infusion from</td>
</tr>
<tr>
<td>Bacto Dextrose</td>
</tr>
<tr>
<td>Bacto Agar</td>
</tr>
<tr>
<td>Final pH 5.6 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>
Section II

Potato Dextrose Broth

Formula per liter
Potatoes, Infusion from . . . . . . . . . . . . . . . . . . . . . . . . . . . 200 g
Bacto Dextrose . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 20 g

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

User Quality Control

Identity Specifications

<table>
<thead>
<tr>
<th>Potato Dextrose Agar</th>
<th>Dehydrated Appearance:</th>
<th>Light beige, homogeneous, free-flowing.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solution:</td>
<td>3.9% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly opalescent.</td>
</tr>
<tr>
<td></td>
<td>Prepared Medium:</td>
<td>Light amber, slightly opalescent.</td>
</tr>
<tr>
<td></td>
<td>Reaction of 3.9% Solution at 25°C:</td>
<td>pH 5.6 ± 0.2</td>
</tr>
</tbody>
</table>

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.
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. |

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>16404</td>
<td>100-1000</td>
<td>good</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10231</td>
<td>100-1000</td>
<td>good</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>9763</td>
<td>100-1000</td>
<td>good</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>9533</td>
<td>undiluted</td>
<td>good</td>
</tr>
</tbody>
</table>

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

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.

Potato Dextrose Broth

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

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>16404</td>
<td>100-1000</td>
<td>good</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10231</td>
<td>100-1000</td>
<td>good</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>9763</td>
<td>100-1000</td>
<td>good</td>
</tr>
</tbody>
</table>
Specimen Collection and Preparation
Refer to appropriate references for specimen collection and preparation.

Test Procedure

Potato Dextrose Agar
Pour plate method
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

Packaging

<table>
<thead>
<tr>
<th></th>
<th>Potato Dextrose Agar</th>
<th>Potato Dextrose Broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>packaging</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 g</td>
<td>500 g</td>
</tr>
<tr>
<td></td>
<td>500 g</td>
<td>10 kg</td>
</tr>
<tr>
<td></td>
<td>2 kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0013-15</td>
<td>0549-17</td>
</tr>
<tr>
<td></td>
<td>0013-17</td>
<td>0549-08</td>
</tr>
<tr>
<td></td>
<td>0013-07</td>
<td></td>
</tr>
</tbody>
</table>

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% CO₂ for up to 72 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucella abortus</td>
<td>4315</td>
<td>100-1,000</td>
<td>Good</td>
</tr>
<tr>
<td>Brucella melitensis</td>
<td>4309</td>
<td>100-1,000</td>
<td>Good</td>
</tr>
<tr>
<td>Brucella suis</td>
<td>4314</td>
<td>100-1,000</td>
<td>Good</td>
</tr>
</tbody>
</table>

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. Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure. Trypsite agar w/ 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae. 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. 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

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
</table>
| 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)

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% CO₂ 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


### 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 ³ ⁴

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 testing¹ and by US EPA.⁵

**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.
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)**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Extract</td>
<td>3 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>5 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>7.46 g</td>
</tr>
<tr>
<td>Tryptose</td>
<td>9.83 g</td>
</tr>
<tr>
<td>Dibasic Phosphate</td>
<td>1.35 g</td>
</tr>
<tr>
<td>Monobasic Phosphate</td>
<td>1.35 g</td>
</tr>
<tr>
<td>Chloride</td>
<td>2.46 g</td>
</tr>
<tr>
<td>Lauryl Sulfate</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Brom Cresol Purple</td>
<td>0.0085 g</td>
</tr>
</tbody>
</table>

**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.

**User Quality Control**

**Identity Specifications**

- **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.

**Cultural Response**

Prepare Presence-Absence Broth in triple strength solution (9.15%). Sterilize 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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM</th>
<th>GROWTH</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>29212*</td>
<td>100-1,000</td>
<td>moderate</td>
<td>slight yellow to purple</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>100-1,000</td>
<td>good</td>
<td>yellow color w/ or w/o gas production</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>13762</td>
<td>100-1,000</td>
<td>good</td>
<td>yellow color w/ or w/o gas production</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>27853*</td>
<td>100-1,000</td>
<td>poor to moderate</td>
<td>no color change</td>
</tr>
</tbody>
</table>

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.

**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
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. 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.
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

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, 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
Incubator (35°C)
Water bath (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.

Cultural Response
Prepare Proteose Agar No. 3 per label directions. Inoculate and incubate at 35 ± 2°C under approximately 5-10% CO₂ for 18-48 hours.

INOCULUM 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.

References
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.

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 Thomas9 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.

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
Typical Analysis

Physical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>PROTEOSE PEPTONE</th>
<th>PROTEOSE PEPTONE NO. 2</th>
<th>PROTEOSE PEPTONE NO. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash (%)</td>
<td>11.1</td>
<td>12.7</td>
<td>11.4</td>
</tr>
<tr>
<td>Clarity, 1% Solution (NTU)</td>
<td>1.4</td>
<td>1.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Filterability (g/cm²)</td>
<td>0.9</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Loss on Drying (%)</td>
<td>3.1</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>pH, 1% Solution</td>
<td>7.2</td>
<td>7.2</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Carbohydrate (%)

<table>
<thead>
<tr>
<th></th>
<th>PROTEOSE PEPTONE</th>
<th>PROTEOSE PEPTONE NO. 2</th>
<th>PROTEOSE PEPTONE NO. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>&lt;0.1</td>
<td>1.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Nitrogen Content (%)

<table>
<thead>
<tr>
<th></th>
<th>PROTEOSE PEPTONE</th>
<th>PROTEOSE PEPTONE NO. 2</th>
<th>PROTEOSE PEPTONE NO. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nitrogen</td>
<td>14.0</td>
<td>12.6</td>
<td>13.2</td>
</tr>
<tr>
<td>Amino Nitrogen</td>
<td>2.9</td>
<td>5.0</td>
<td>3.5</td>
</tr>
<tr>
<td>AN/TN</td>
<td>20.7</td>
<td>39.7</td>
<td>26.5</td>
</tr>
</tbody>
</table>

Amino Acids (%)

<table>
<thead>
<tr>
<th></th>
<th>PROTEOSE PEPTONE</th>
<th>PROTEOSE PEPTONE NO. 2</th>
<th>PROTEOSE PEPTONE NO. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>6.50</td>
<td>6.08</td>
<td>5.99</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.12</td>
<td>5.47</td>
<td>5.49</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>7.28</td>
<td>7.45</td>
<td>6.92</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.87</td>
<td>0.40</td>
<td>1.12</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>11.95</td>
<td>10.57</td>
<td>12.38</td>
</tr>
<tr>
<td>Glycine</td>
<td>9.68</td>
<td>10.84</td>
<td>9.26</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.01</td>
<td>&lt;0.01</td>
<td>1.74</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.04</td>
<td>1.00</td>
<td>2.65</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.66</td>
<td>3.57</td>
<td>5.70</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.33</td>
<td>5.22</td>
<td>5.02</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.97</td>
<td>1.51</td>
<td>1.86</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.86</td>
<td>7.94</td>
<td>2.72</td>
</tr>
<tr>
<td>Proline</td>
<td>5.93</td>
<td>5.31</td>
<td>4.94</td>
</tr>
<tr>
<td>Serine</td>
<td>3.49</td>
<td>4.64</td>
<td>3.65</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.14</td>
<td>3.90</td>
<td>3.32</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.60</td>
<td>0.94</td>
<td>0.59</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.35</td>
<td>1.92</td>
<td>1.96</td>
</tr>
<tr>
<td>Valine</td>
<td>3.76</td>
<td>4.73</td>
<td>3.62</td>
</tr>
</tbody>
</table>

Inorganics (%)

<table>
<thead>
<tr>
<th></th>
<th>PROTEOSE PEPTONE</th>
<th>PROTEOSE PEPTONE NO. 2</th>
<th>PROTEOSE PEPTONE NO. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.021</td>
<td>0.024</td>
<td>0.023</td>
</tr>
<tr>
<td>Chloride</td>
<td>4.510</td>
<td>3.644</td>
<td>3.581</td>
</tr>
<tr>
<td>Cobalt</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Copper</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iron</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.027</td>
<td>0.024</td>
<td>0.027</td>
</tr>
<tr>
<td>Manganese</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.872</td>
<td>1.674</td>
<td>1.447</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.685</td>
<td>0.815</td>
<td>0.982</td>
</tr>
<tr>
<td>Sodium</td>
<td>3.677</td>
<td>3.956</td>
<td>3.815</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0.162</td>
<td>0.232</td>
<td>0.232</td>
</tr>
<tr>
<td>Sulfur</td>
<td>0.812</td>
<td>0.698</td>
<td>0.975</td>
</tr>
<tr>
<td>Tin</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.002</td>
<td>0.003</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Vitamins (µg/g)

<table>
<thead>
<tr>
<th></th>
<th>PROTEOSE PEPTONE</th>
<th>PROTEOSE PEPTONE NO. 2</th>
<th>PROTEOSE PEPTONE NO. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>0.1</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Choline</td>
<td>2300.0</td>
<td>4500.0</td>
<td>3700.0</td>
</tr>
<tr>
<td>Cyanocobalamin</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>0.4</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Inositol</td>
<td>5000.0</td>
<td>4700.0</td>
<td>8900.0</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>79.9</td>
<td>157.1</td>
<td>124.2</td>
</tr>
<tr>
<td>PABA</td>
<td>4.2</td>
<td>1.2</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>20.0</td>
<td>47.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>1.1</td>
<td>4.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>&lt;0.1</td>
<td>6.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Thiamine</td>
<td>1.2</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Thymidine</td>
<td>99.7</td>
<td>1319.0</td>
<td>659.6</td>
</tr>
</tbody>
</table>

Biological Testing (CFU/g)

<table>
<thead>
<tr>
<th></th>
<th>PROTEOSE PEPTONE</th>
<th>PROTEOSE PEPTONE NO. 2</th>
<th>PROTEOSE PEPTONE NO. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Salmonella</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Spore Count</td>
<td>393</td>
<td>75</td>
<td>890</td>
</tr>
<tr>
<td>Standard Plate Count</td>
<td>443</td>
<td>1450</td>
<td>915</td>
</tr>
<tr>
<td>Thermophile Count</td>
<td>73</td>
<td>&lt;50</td>
<td>25</td>
</tr>
</tbody>
</table>

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 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

<table>
<thead>
<tr>
<th></th>
<th>PROTEOSE PEPTONE</th>
<th>PROTEOSE PEPTONE NO. 2</th>
<th>PROTEOSE PEPTONE NO. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>6.50</td>
<td>6.08</td>
<td>5.99</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.12</td>
<td>5.47</td>
<td>5.49</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>7.28</td>
<td>7.45</td>
<td>6.92</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.87</td>
<td>0.40</td>
<td>1.12</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>11.95</td>
<td>10.57</td>
<td>12.38</td>
</tr>
<tr>
<td>Glycine</td>
<td>9.68</td>
<td>10.84</td>
<td>9.26</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.01</td>
<td>&lt;0.01</td>
<td>1.74</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.04</td>
<td>1.00</td>
<td>2.65</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.66</td>
<td>3.57</td>
<td>5.70</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.33</td>
<td>5.22</td>
<td>5.02</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.97</td>
<td>1.51</td>
<td>1.86</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.86</td>
<td>7.94</td>
<td>2.72</td>
</tr>
<tr>
<td>Proline</td>
<td>5.93</td>
<td>5.31</td>
<td>4.94</td>
</tr>
<tr>
<td>Serine</td>
<td>3.49</td>
<td>4.64</td>
<td>3.65</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.14</td>
<td>3.90</td>
<td>3.32</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.60</td>
<td>0.94</td>
<td>0.59</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.35</td>
<td>1.92</td>
<td>1.96</td>
</tr>
<tr>
<td>Valine</td>
<td>3.76</td>
<td>4.73</td>
<td>3.62</td>
</tr>
</tbody>
</table>

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.
Section II Proteose Peptones

Procedure

Materials Provided

- Proteose Peptone
- Proteose Peptone No. 2
- Proteose Peptone No. 3
- H₂S 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


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.

<table>
<thead>
<tr>
<th>TEST SOLUTION ORGANISM</th>
<th>ATCC</th>
<th>INOCULUM</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentable Carbohydrate 2%</td>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>1 drop, undiluted negative; red color</td>
</tr>
<tr>
<td>Indole Production 0.1%</td>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>1 drop, undiluted positive; pink color on Indole Test Strip</td>
</tr>
<tr>
<td>Acetyl-methylcarbinol Production (AMC) 0.1% w/ 0.5% dextrose</td>
<td><em>Enterobacter aerogenes</em></td>
<td>13048*</td>
<td>1 drop, undiluted positive; pink color upon adding reagents</td>
</tr>
<tr>
<td>Hydrogen Sulfide Production 1%</td>
<td><em>Salmonella typhi</em></td>
<td>6539</td>
<td>1 drop, undiluted positive; brownish blackening of H₂S Test Strip</td>
</tr>
<tr>
<td>Growth Response 2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose</td>
<td><em>Brucella suis</em></td>
<td>4314</td>
<td>undiluted good growth</td>
</tr>
<tr>
<td>Growth Response 2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose</td>
<td><em>Staphylococcus aureus</em></td>
<td>25923*</td>
<td>100-1,000 CFU good growth</td>
</tr>
<tr>
<td>Growth Response 2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose</td>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>100-1,000 CFU good growth</td>
</tr>
</tbody>
</table>

### 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.

<table>
<thead>
<tr>
<th>TEST</th>
<th>ORGANISM</th>
<th>ATCC</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxin Production</td>
<td><em>Corynebacterium diphtheriae</em> Type intermedius</td>
<td>8032</td>
<td>precipitin line</td>
</tr>
<tr>
<td>Toxin Production</td>
<td><em>Corynebacterium diphtheriae</em> Type gravis</td>
<td>8028</td>
<td>precipitin line</td>
</tr>
<tr>
<td>Toxin Production</td>
<td><em>Corynebacterium diphtheriae</em> Type mitis</td>
<td>8024</td>
<td>precipitin line</td>
</tr>
</tbody>
</table>

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.*

### Packaging

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose Peptone</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Proteose Peptone No. 2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Proteose Peptone No. 3</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
**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.

**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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC®</th>
<th>Growth</th>
<th>Pseudomonas Agar F Pigment Production</th>
<th>Pseudomonas Agar P Pigment Production</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>9027</td>
<td>good</td>
<td>greenish yellow</td>
<td>blue</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>27853*</td>
<td>good</td>
<td>greenish yellow</td>
<td>blue</td>
</tr>
<tr>
<td><em>Pseudomonas cepacia</em></td>
<td>25609</td>
<td>good</td>
<td>no pigment</td>
<td>no pigment</td>
</tr>
</tbody>
</table>

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.

**Summary and Explanation**
Pseudomonas Agar F and Pseudomonas Agar P, patterned after the formulations described by King, Ward and Raney, are modified to USP specifications.

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...
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.1

**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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone . .</td>
<td>10 g</td>
</tr>
<tr>
<td>Bacto Proteose Peptone No. 3. .</td>
<td>10 g</td>
</tr>
<tr>
<td>Dipotassium Phosphate .</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Magnesium Sulfate .</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Bacto Agar . . . . . . . . . . .</td>
<td>15 g</td>
</tr>
<tr>
<td>Final pH 7.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

**Pseudomonas Agar P**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Peptone . . .</td>
<td>20 g</td>
</tr>
<tr>
<td>Magnesium Chloride .</td>
<td>1.4 g</td>
</tr>
<tr>
<td>Potassium Sulfate .</td>
<td>10 g</td>
</tr>
<tr>
<td>Bacto Agar . . . . . . . . .</td>
<td>15 g</td>
</tr>
<tr>
<td>Final pH 7.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

**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 (CHCl₃).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**


**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. 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. It is especially useful for isolating *Pseudomonas* from clinical specimens such as stools, wounds and urine. Pseudomonas Isolation Agar includes Irgasan®, a potent broad spectrum antimicrobial that is not active against *Pseudomonas*. 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.

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM</th>
<th>GROWTH</th>
<th>APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>marked to complete inhibition</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10145</td>
<td>100-1,000</td>
<td>good</td>
<td>green to blue-green</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>27853*</td>
<td>100-1,000</td>
<td>good</td>
<td>green to blue-green</td>
</tr>
</tbody>
</table>

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.

**Packaging**
- Pseudomonas Agar F
  - 100 g 0448-15
- Pseudomonas Agar P
  - 500 g 0448-17

**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**

<table>
<thead>
<tr>
<th>Pseudomonas Isolation Agar</th>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Peptone</td>
<td>20 g</td>
</tr>
<tr>
<td>Magnesium Chloride</td>
<td>1.4 g</td>
</tr>
<tr>
<td>Potassium Sulfate</td>
<td>10 g</td>
</tr>
<tr>
<td>Irgasan®</td>
<td>0.025 g</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>13.6 g</td>
</tr>
<tr>
<td>Final pH 7.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>
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

Packaging

<table>
<thead>
<tr>
<th>Component</th>
<th>Package Size</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas Isolation Agar</td>
<td>500 g</td>
<td>0927-17</td>
</tr>
<tr>
<td>Glycerol</td>
<td>100 g</td>
<td>0282-15</td>
</tr>
<tr>
<td></td>
<td>500 g</td>
<td>0282-17</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>REACTION w/1% DEXTROSE ACID</th>
<th>GAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcaligenes faecalis</td>
<td>8750</td>
<td>1,000-2,000</td>
<td>good</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>good</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>14028*</td>
<td>1,000-2,000</td>
<td>good</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>REACTION w/1% DEXTROSE ACID</th>
<th>GAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcaligenes faecalis</td>
<td>8750</td>
<td>1,000-2,000</td>
<td>good</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>good</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>14028*</td>
<td>1,000-2,000</td>
<td>good</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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.
Section II

Purple Broth Base & 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

Packaging
Purple Broth Base 500 g 0227-17
Purple Agar Base 500 g 0228-17
**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. 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. 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.

**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**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Beef Extract</td>
<td>3 g</td>
</tr>
<tr>
<td>Bacto Peptone</td>
<td>5 g</td>
</tr>
<tr>
<td>Bacto Lactose</td>
<td>10 g</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>10 g</td>
</tr>
<tr>
<td>Bacto Brom Cresol Purple</td>
<td>0.025 g</td>
</tr>
<tr>
<td><strong>Final pH</strong></td>
<td>6.8 ± 0.1 at 25°C</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC</th>
<th>Growth</th>
<th>Acid (Yellow)</th>
<th>Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter aerogenes</td>
<td>13048*</td>
<td>good</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>good</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>19430</td>
<td>good</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923*</td>
<td>good</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

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**
Purple Lactose Agar

**Materials Required But Not Provided**
Glassware
Autoclave
Incubator

---

Image: 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, Clinical Microbiology Procedures Handbook and Bailey & Scott’s Diagnostic Microbiology.

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.

References

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:

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
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
Sterile tubes

Materials Required But Not Provided

Pyridoxine Y Medium

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.

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 IRITATING 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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin</td>
<td>20 µg</td>
</tr>
<tr>
<td>Inositol</td>
<td>5 mg</td>
</tr>
<tr>
<td>Boric Acid</td>
<td>200 µg</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>3 g</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>1 g</td>
</tr>
<tr>
<td>Ammonium Sulfate</td>
<td>4 g</td>
</tr>
<tr>
<td>Calcium Chloride</td>
<td>0.49 g</td>
</tr>
<tr>
<td>Potassium Iodide</td>
<td>200 µg</td>
</tr>
<tr>
<td>Ammonium Molybdate</td>
<td>40 µg</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>80 µg</td>
</tr>
<tr>
<td>Copper Sulfate</td>
<td>90 µg</td>
</tr>
<tr>
<td>Zinc Sulfate</td>
<td>80 µg</td>
</tr>
<tr>
<td>Ferrous Sulfate</td>
<td>500 µg</td>
</tr>
</tbody>
</table>

Final pH 4.4 ± 0.2 at 25°C
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**

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

**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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Yeast Extract</td>
</tr>
<tr>
<td>Bacto Proteose Peptone No. 3</td>
</tr>
<tr>
<td>Bacto Casamino Acids</td>
</tr>
<tr>
<td>Bacto Dextrose</td>
</tr>
<tr>
<td>Soluble Starch</td>
</tr>
<tr>
<td>Sodium Pyruvate</td>
</tr>
<tr>
<td>Potassium Phosphate, Dibasic</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
</tr>
<tr>
<td>Bacto Agar</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Minimum Incubation Time</th>
<th>Optimal Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>35°C</td>
<td>72 hours</td>
<td>5-7 days</td>
</tr>
<tr>
<td>20 or 28°C</td>
<td>5 days</td>
<td>7 days</td>
</tr>
</tbody>
</table>

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

Packaging

<table>
<thead>
<tr>
<th>R2A Agar</th>
<th>100 g</th>
<th>1826-15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 g</td>
<td>1826-17</td>
</tr>
<tr>
<td></td>
<td>2 kg</td>
<td>1826-07</td>
</tr>
</tbody>
</table>
### 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 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.

**User Quality Control**

**Identity Specifications**

<table>
<thead>
<tr>
<th>Raka-Ray No. 3 Broth</th>
<th>Dehydrated Appearance:</th>
<th>Beige, free-flowing, homogeneous.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution:</td>
<td></td>
<td>5.89% solution, soluble in distilled or deionized water with 1% Tween® 80. Solution is medium to dark amber, clear.</td>
</tr>
<tr>
<td>Reaction of 5.89%</td>
<td></td>
<td>pH 5.4 ± 0.2</td>
</tr>
<tr>
<td>Solution at 25°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Raka-Ray No. 3 Medium</th>
<th>Dehydrated Appearance:</th>
<th>Beige, free-flowing, homogeneous.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution:</td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Reaction of 7.49%</td>
<td></td>
<td>pH 5.4 ± 0.2</td>
</tr>
<tr>
<td>Solution at 25°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC #</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>none to poor</td>
</tr>
<tr>
<td><em>Lactobacillus brevis</em></td>
<td>367</td>
<td>30-300</td>
<td>good</td>
</tr>
<tr>
<td><em>Lactobacillus buchneri</em></td>
<td>11307</td>
<td>30-300</td>
<td>good</td>
</tr>
<tr>
<td><em>Pediococcus acidilactici</em></td>
<td>8042</td>
<td>30-300</td>
<td>good</td>
</tr>
</tbody>
</table>

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.*

Agar, W-L Differential Agar and Universal Beer Agar, with larger colonies developing after 2-4 days of anaerobic incubation.

Raka-Ray No. 3 Medium yields larger lactic acid bacterial colonies than Universal Beer Agar. 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.

Raka-Ray No. 3 Medium is also recommended by the ‘European Brewing Congress Analytical Microbiologica’ for enumeration of lactobacilli and pediococci. 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 aceton per liter of medium before autoclaving. Yeasts and gram-negative bacteria are suppressed, facilitating enumeration of the lactic bacterial flora.

**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 gram-negative bacteria.

**Formula**

**Raka-Ray No. 3 Broth**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Yeast Extract</td>
</tr>
<tr>
<td>Bacto Tryptone</td>
</tr>
<tr>
<td>Liver Digest</td>
</tr>
<tr>
<td>Maltose Reagent</td>
</tr>
<tr>
<td>Fructose</td>
</tr>
<tr>
<td>Dextrose</td>
</tr>
<tr>
<td>Betaine Hydrochloride</td>
</tr>
<tr>
<td>Di-ammonium Citrate</td>
</tr>
<tr>
<td>L-Aspartic Acid</td>
</tr>
<tr>
<td>Magnesium Sulphate</td>
</tr>
<tr>
<td>Manganese Sulphate</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
</tr>
<tr>
<td>N-Acetyl Glucosamine</td>
</tr>
<tr>
<td>Potassium Glutamate</td>
</tr>
<tr>
<td>Final pH 5.4 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

**Raka-Ray No. 3 Medium**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Yeast Extract</td>
</tr>
<tr>
<td>Bacto Tryptone</td>
</tr>
<tr>
<td>Liver Digest</td>
</tr>
<tr>
<td>Maltose Reagent</td>
</tr>
<tr>
<td>Fructose</td>
</tr>
<tr>
<td>Dextrose</td>
</tr>
<tr>
<td>Betaine Hydrochloride</td>
</tr>
<tr>
<td>Di-ammonium Citrate</td>
</tr>
<tr>
<td>L-Aspartic Acid</td>
</tr>
<tr>
<td>Magnesium Sulphate</td>
</tr>
<tr>
<td>Manganese Sulphate</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
</tr>
<tr>
<td>N-Acetyl Glucosamine</td>
</tr>
<tr>
<td>Potassium Glutamate</td>
</tr>
<tr>
<td>Bacto Agar</td>
</tr>
<tr>
<td>Final pH 5.4 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>
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 (H₂/CO₂) 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

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

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.

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptose</td>
<td>4.59 g</td>
</tr>
<tr>
<td>Casein Hydrolysate (Acid)</td>
<td>4.59 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>7.34 g</td>
</tr>
<tr>
<td>Potassium Dihydrogen Phosphate</td>
<td>1.47 g</td>
</tr>
<tr>
<td>Magnesium Chloride Anhydrous</td>
<td>10.93 g</td>
</tr>
<tr>
<td>Malachite Green Oxalate</td>
<td>0.037 g</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>2.7 g</td>
</tr>
<tr>
<td>Final pH 5.2 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

Novobiocin Antimicrobic Supplement

Formula per 10 ml

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Novobiocin</td>
<td>20 mg</td>
</tr>
</tbody>
</table>

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...
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 Antimicrobial 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 Antimicrobial 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 Antimicrobial 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

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.
2. Incubate at 35°C for 20 ± 2 hours.

Selective Enrichment

3. Inoculate 1 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

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:

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


Packaging

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

*Store at 2-8°C

FDA

The Difco Manual
**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. formulated an enrichment medium for *Salmonella* that was modified by Vassiliadis et al. 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.

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, raw flesh foods, highly contaminated foods and animal feeds.

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**

<table>
<thead>
<tr>
<th>Identity Specifications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dehydrated Appearance:</strong></td>
<td>Pale green to green, free-flowing, homogeneous.</td>
</tr>
<tr>
<td><strong>Solution:</strong></td>
<td>2.66% solution, soluble in distilled or deionized water upon gentle heating; blue, clear.</td>
</tr>
<tr>
<td><strong>Reaction of 2.66% Solution at 25°C:</strong></td>
<td>pH 5.1 ± 0.2</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>markedly inhibited</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>13076</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>14028*</td>
<td>100-1,000</td>
<td>good</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th><strong>Formula Per Liter</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone</td>
<td>4.54 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>7.2 g</td>
</tr>
<tr>
<td>Potassium Dihydrogen Phosphate</td>
<td>1.45 g</td>
</tr>
<tr>
<td>Magnesium Chloride Anhydrous</td>
<td>13.4 g</td>
</tr>
<tr>
<td>Malachite Green Oxalate</td>
<td>0.036 g</td>
</tr>
</tbody>
</table>

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.

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

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 hours5,6 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 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

Packaging
Rappaport-Vassiliadis R10 Broth 500 g 1858-17
used the medium to dilute vegetative cells of *Clostridium perfringens*. Barnes et al. 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.

**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

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptose</td>
<td>10 g</td>
<td></td>
</tr>
<tr>
<td>Bacto Beef Extract</td>
<td>10 g</td>
<td></td>
</tr>
<tr>
<td>Bacto Yeast Extract</td>
<td>3 g</td>
<td></td>
</tr>
<tr>
<td>Bacto Dextrose</td>
<td>5 g</td>
<td></td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5 g</td>
<td></td>
</tr>
<tr>
<td>Soluble Starch</td>
<td>1 g</td>
<td></td>
</tr>
<tr>
<td>Cysteine Hydrochloride</td>
<td>0.5 g</td>
<td></td>
</tr>
<tr>
<td>Sodium Acetate</td>
<td>3 g</td>
<td></td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>0.5 g</td>
<td></td>
</tr>
</tbody>
</table>

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**

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**


**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 used for determining riboflavin concentration by the microbiological assay technique.

Principles of the Procedure
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 Administration2 using Lactobacillus casei subsp. rhamnosus ATCC® 7469 as the test organism.

Formula

<table>
<thead>
<tr>
<th>riboflavin assay medium</th>
<th>formula per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacto dextrose</td>
<td>20 g</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>15 g</td>
</tr>
<tr>
<td>Bacto Vitamin Assay Casamino Acids</td>
<td>10 g</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>1 g</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>1 g</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>0.6 g</td>
</tr>
<tr>
<td>DL-Tryptophane</td>
<td>0.2 g</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Adenine Sulfate</td>
<td>20 mg</td>
</tr>
<tr>
<td>Guanine Hydrochloride</td>
<td>20 mg</td>
</tr>
<tr>
<td>Uracil</td>
<td>20 mg</td>
</tr>
<tr>
<td>Xanthine</td>
<td>20 mg</td>
</tr>
<tr>
<td>Magnesium Sulfate USP</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Ferrous Sulfate</td>
<td>20 mg</td>
</tr>
<tr>
<td>Manganese Sulfate Monohydrate</td>
<td>20 mg</td>
</tr>
<tr>
<td>Sodium Chloride USP</td>
<td>20 mg</td>
</tr>
<tr>
<td>Pyridoxine Hydrochloride</td>
<td>4 mg</td>
</tr>
<tr>
<td>Pyridoxal Hydrochloride</td>
<td>4 mg</td>
</tr>
<tr>
<td>p-Aminobenzoic Acid</td>
<td>2 mg</td>
</tr>
<tr>
<td>Calcium Pantothenate</td>
<td>800 µg</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>800 µg</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>800 µg</td>
</tr>
<tr>
<td>Thiamine Hydrochloride</td>
<td>400 µg</td>
</tr>
<tr>
<td>Biotin</td>
<td>1 µg</td>
</tr>
</tbody>
</table>

Final pH 6.8 ± 0.2 at 25°C

Precautions
1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Take great care to avoid contamination of media or glassware for identity and performance.
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. 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
2. Association of Analytical Chemists. 1996. U.S. Food and Drug Administration methods or the microbiological analysis of selected nutrients. AOAC International, Gaithersburg, MD.

Packaging
Riboflavin Assay Medium 100 g 0325-15*
*Store at 2-8°C
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

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>White Rice, Extract from ........................</td>
<td>20 g</td>
</tr>
<tr>
<td>Bacto Agar ..................</td>
<td>20 g</td>
</tr>
<tr>
<td>Final pH 7.1 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>INOCULUM</th>
<th>CFU</th>
<th>GROWTH</th>
<th>CHLAMYDOSPORES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>10231</td>
<td>30-300</td>
<td>good</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>26790</td>
<td>30-300</td>
<td>good</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

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
Auto clave
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.

**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.
3. High temperatures for incubation should be avoided as chlamydospores are not formed at 37°C.

**References**


**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. These media are used for isolation, enumeration and identification of lactobacilli in oral bacteriology, feces, vaginal specimens and foodstuffs. The low pH and high acetate concentrations effectively suppress other bacterial flora allowing lactobacilli to flourish.

**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
- Bacto Agar: 15 g

**Final pH 5.4 ± 0.2 at 25°C**

**User Quality Control**

<table>
<thead>
<tr>
<th>Identity Specifications</th>
<th>Rogosa SL Agar</th>
<th>Rogosa SL Broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated Appearance:</td>
<td>Beige, homogeneous with soft clumps.</td>
<td>Beige, appears moist, slightly lumpy.</td>
</tr>
<tr>
<td>Solution:</td>
<td>7.5% solution, soluble in distilled or deionized water upon boiling.</td>
<td>6.0% solution, soluble in distilled or deionized water upon boiling.</td>
</tr>
<tr>
<td>Reaction of 7.5%</td>
<td>pH 5.4 ± 0.2 at 25°C</td>
<td>pH 5.4 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

**Cultural Response**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>INOCULUM</th>
<th>GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus casei</td>
<td>9595</td>
<td>100-1,000</td>
</tr>
<tr>
<td>Lactobacillus delbrueckii</td>
<td>4797</td>
<td>100-1,000</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923*</td>
<td>1,000-2,000</td>
</tr>
</tbody>
</table>

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.

**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

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 Antimicrobial Supplement C

Intended Use
Bacto Rose Bengal Agar Base is used with Bacto Rose Bengal Antimicrobial 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,4 and have little effect in restricting the size of mold colonies.4 Smith and Dawson5 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.6,7,8,9,10,11

Rose Bengal Agar Base supplemented with Rose Bengal Antimicrobial Supplement C is a modification of the Rose Bengal Chlorotetrcycline 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.10 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 Antimicrobial Supplement C is a lyophilized antimicrobial 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 Antimicrobial Supplement C**

Formula Per 2 ml Vial

- Chloramphenicol .................. 0.05 g

**Precautions**

1. For Laboratory Use.

2. **Rose Bengal Antimicrobial 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 Antimicrobial 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 Antimicrobial 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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>COLONY COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>1015</td>
<td>100-300</td>
<td>good</td>
<td>white</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10231</td>
<td>100-300</td>
<td>good</td>
<td>pink</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25923</td>
<td>1,000-2,000</td>
<td>inhibited</td>
<td>–</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>10240</td>
<td>1,000-2,000</td>
<td>inhibited</td>
<td>–</td>
</tr>
</tbody>
</table>

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 Antimicrobial 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 Antimicrobial 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 Antimicrobial 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. Prepare samples for dilution plating inoculation. It is recommended that the laboratory test procedure should be taken not to expose this medium to light since photo-degradation of rose bengal yields compounds that are toxic to fungi.

**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 photo-degradation of rose bengal yields compounds that are toxic to fungi.

**References**

**Packaging**
- Rose Bengal Agar Base 500 g 1831-17
- 10 kg 1831-08
- Rose Bengal Antimicrobial 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 Dextrose 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**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf Brains, Infusion from</td>
<td>100 g</td>
</tr>
<tr>
<td>Beef Heart, Infusion from</td>
<td>125 g</td>
</tr>
<tr>
<td>Bacto Proteose Peptone</td>
<td>5 g</td>
</tr>
<tr>
<td>Bacto Neopeptone</td>
<td>5 g</td>
</tr>
<tr>
<td>Bacto Dextrose</td>
<td>21 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Disodium Phosphate</td>
<td>1.25 g</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>15 g</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC</th>
<th>Inoculum (CFU)</th>
<th>Growth with Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>16404</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>10231</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>marked to complete inhibition</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>9763</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>marked to complete inhibition</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>9533</td>
<td>100-1,000</td>
<td>good</td>
</tr>
</tbody>
</table>

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.

**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.

**References**

**Packaging**
SABHI Agar Base

<table>
<thead>
<tr>
<th>Packaging</th>
<th>SABHI Agar Base</th>
<th>500 g</th>
<th>0797-17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 kg</td>
<td>0797-07</td>
<td></td>
</tr>
</tbody>
</table>

**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 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. 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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone</td>
</tr>
<tr>
<td>Bacto Dextrose</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
</tr>
</tbody>
</table>
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.

**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**

<table>
<thead>
<tr>
<th>Dehydrated Appearance:</th>
<th>Light beige to gray, may have a light greenish tint, free-flowing, homogeneous.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution:</td>
<td>3.6% solution, soluble in distilled or deionized water. Solution is purple, clear with no precipitate.</td>
</tr>
<tr>
<td>Prepared Tubes:</td>
<td>Purple, clear with no precipitate.</td>
</tr>
<tr>
<td>Reaction of 3.6% Solution at 25°C:</td>
<td>pH 6.9 ± 0.2</td>
</tr>
</tbody>
</table>

**Cultural Response**

Prepare SF Medium per label directions. Inoculate and incubate at 45 ± 0.5°C 18-24 and 40-48 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM</th>
<th>CFU</th>
<th>GROWTH</th>
<th>ACID REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>19433*</td>
<td>1,000-2,000</td>
<td>good</td>
<td>yellow (acid)</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>27270</td>
<td>1,000-2,000</td>
<td>good</td>
<td>yellow (acid)</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>inhibited</td>
<td>no change</td>
<td></td>
</tr>
<tr>
<td>Streptococcus bovis</td>
<td>33317</td>
<td>1,000-2,000</td>
<td>none to</td>
<td>no change</td>
<td></td>
</tr>
</tbody>
</table>

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.

**References**

SFP Agar

Bacto SFP Agar Base · Bacto Egg Yolk Enrichment 50% · Bacto Antimicrobial Vial K · Bacto Antimicrobial Vial P

Intended Use
Bacto SFP Agar Base is used with Bacto Egg Yolk Enrichment 50%, Bacto Antimicrobial Vial P and Bacto Antimicrobial 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. 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 polymyxin 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.

**Antimicrobial Vial K**
- Dehydrated Appearance: White cake or powder.
- Rehydrated Appearance: Colorless, clear solution.

**Antimicrobial 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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM (CFU)</th>
<th>GROWTH</th>
<th>COLOR OF COLONIES</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>12919</td>
<td>30-300</td>
<td>good</td>
<td>black with halo</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>12924</td>
<td>30-300</td>
<td>good</td>
<td>black with halo</td>
</tr>
</tbody>
</table>

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. Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated. Enumerating the microorganism in food samples plays a role in the epidemiological investigation of outbreaks of food borne illness.

SFP Agar (with added kanamycin and polymyxin B) is comparable to Tryptose Sulfite Cycloserine (TSC) Agar, which uses cycloserine as the inhibitory component. 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 H2S 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.

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 H2S 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

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Yeast Extract</td>
<td>5</td>
</tr>
<tr>
<td>Bacto Tryptose</td>
<td>15</td>
</tr>
<tr>
<td>Bacto Soytone</td>
<td>5</td>
</tr>
<tr>
<td>Ferric Ammonium Citrate</td>
<td>1</td>
</tr>
<tr>
<td>Sodium Bisulfite</td>
<td>1</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>20</td>
</tr>
</tbody>
</table>

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. **Antimicrobial Vial K**
   HARMFUL. MAY BE 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.

**Antimicrobial 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 Antimicrobial Vial P (30,000 units polymyxin B sulfate) and 4.8 ml rehydrated Antimicrobial 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.

**Antimicrobial Vial K**
1. Aseptically add 10 ml sterile distilled or deionized water to the Antimicrobial Vial K.
2. Shake to dissolve contents.

**Antimicrobial Vial P**
1. Aseptically add 10 ml sterile distilled or deionized water to the Antimicrobial 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.

---

**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. 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 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 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.

**References**

**Formula**

**Nitrate Broth**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 g</td>
</tr>
<tr>
<td>3 g</td>
</tr>
<tr>
<td>0.2 g</td>
</tr>
<tr>
<td>0.02 g</td>
</tr>
<tr>
<td>3 g</td>
</tr>
<tr>
<td>Final pH 7.3 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

**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
The Difco Manual

Section II SIM Medium

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, H₂S production and motility. Add 3-4 drops of SpotTest Indole Reagent Kovacs. Indole production is indicated by 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


Packaging

SIM Medium 500 g 0271-17

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, H₂S and Indole production.
4. Add 3-4 drops of SpotTest Indole Reagent Kovacs.

Results

Motility and H₂S 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. H₂S 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.

U tility R eferences

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>GROWTH</th>
<th>H₂S</th>
<th>MOTILITY</th>
<th>INDOLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>good</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>14028*</td>
<td>good</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>6539</td>
<td>good</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>12022*</td>
<td>good</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

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

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

Intended Use
Bacto SOB Medium is used for cultivating recombinant strains of Escherichia coli.

Summary and Explanation
SOB Medium was developed by Hanahan¹ 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

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone</td>
<td>20 g</td>
</tr>
<tr>
<td>Bacto Yeast Extract</td>
<td>5 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>2.4 g</td>
</tr>
<tr>
<td>Potassium Chloride</td>
<td>0.186 g</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM</th>
<th>CFU</th>
<th>GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>53868</td>
<td>100-300</td>
<td></td>
<td>Good</td>
</tr>
</tbody>
</table>

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

Precautions
1. For Laboratory Use.
2. MAY BE IRITATING 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 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

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, Mossel1 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.6

**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 H2S 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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone</td>
</tr>
<tr>
<td>Bacto Yeast Extract</td>
</tr>
<tr>
<td>Ferric Citrate</td>
</tr>
<tr>
<td>Sodium Sulfite</td>
</tr>
<tr>
<td>Sodium Thioglycollate</td>
</tr>
<tr>
<td>Tween® 80</td>
</tr>
<tr>
<td>Sulfadiazine</td>
</tr>
<tr>
<td>Polymyxin B Sulfate</td>
</tr>
<tr>
<td>Bacto Agar</td>
</tr>
<tr>
<td>Final pH 7.0 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
<th>APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>12919</td>
<td>100-1,000</td>
<td>good</td>
<td>black colonies</td>
</tr>
<tr>
<td><em>Clostridium sporogenes</em></td>
<td>11437</td>
<td>100-1,000</td>
<td>none</td>
<td>black colonies</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>100-1,000</td>
<td>marked to fair</td>
<td>complete inhibition</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>14028</td>
<td>100-1,000</td>
<td>marked to fair</td>
<td>complete inhibition</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923</td>
<td>100-1,000</td>
<td>fair</td>
<td>white colonies</td>
</tr>
</tbody>
</table>

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.  

References

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. 5, 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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
<th>APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>29212*</td>
<td>1,000-2,000</td>
<td>partial inhibition</td>
<td>colorless</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>1,000-2,000</td>
<td>partial inhibition</td>
<td>pink to red colorless w/black centers</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>14028*</td>
<td>100-1,000</td>
<td>good</td>
<td>colorless</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>12022*</td>
<td>100-1,000</td>
<td>fair to good</td>
<td>colorless</td>
</tr>
</tbody>
</table>

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
2. Process each specimen, using procedures appropriate for that specimen or sample.1,7

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-fermenters and form colorless colonies on SS Agar. *Salmonella* spp. that are H₂S 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 H₂S 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.*1,7 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

Packaging
<table>
<thead>
<tr>
<th>SS Agar</th>
<th>100 g</th>
<th>0074-15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 g</td>
<td>0074-17</td>
</tr>
<tr>
<td></td>
<td>2 kg</td>
<td>0074-07</td>
</tr>
<tr>
<td></td>
<td>10 kg</td>
<td>0074-08</td>
</tr>
</tbody>
</table>

---

**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 5.6 ± 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 amber, clear.

Prepared Medium: Light amber, clear.

Reaction of 6.5% 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 Dextrose 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

**Sabouraud Maltose Broth**

Dehydrated Appearance: Light amber, clear to slightly opalescent.

Solution at 25°C: pH 5.6 ± 0.2

---

**Summary and Explanation**

Sabouraud Agar Modified is a modification of the Sabouraud Dextrose Agar formulation devised by Raymond Sabouraud for his dermatophyte studies. Sabouraud Agar Modified, used for the recovery of dermatophytes, 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. Sabouraud Dextrose Agar and Sabouraud Dextrose Broth are modifications of the Dextrose Agar described by Sabouraud. 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. Georg 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 and for the mycological evaluation of food. 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 reported that Sabouraud Maltose Agar was a satisfactory medium in their studies of the infections caused by Microsporum audouinii, M. lanoosum and Trichophyton gypseum. Davidson and Dawding 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 acidic 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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Neopeptone</td>
<td>10 g</td>
</tr>
<tr>
<td>Bacto Dextrose</td>
<td>20 g</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>20 g</td>
</tr>
<tr>
<td>Final pH 7.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>
**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.

**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 Medium, 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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>16404</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10231</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>9763</td>
<td>100-1,000</td>
<td>good</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>16404</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10231</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>9763</td>
<td>100-1,000</td>
<td>good</td>
</tr>
</tbody>
</table>

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

- Loose 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

Packaging

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabouraud Agar Modified</td>
<td>500 g</td>
<td>0747-17</td>
</tr>
<tr>
<td></td>
<td>2 kg</td>
<td>0747-07</td>
</tr>
<tr>
<td>Sabouraud Dextrose Agar</td>
<td>50 g</td>
<td>0109-17</td>
</tr>
<tr>
<td></td>
<td>100 g</td>
<td>0109-15</td>
</tr>
<tr>
<td></td>
<td>10 kg</td>
<td>0109-08</td>
</tr>
<tr>
<td></td>
<td>2 kg</td>
<td>0109-07</td>
</tr>
<tr>
<td></td>
<td>10 x 200 ml</td>
<td>9074-76</td>
</tr>
<tr>
<td>Sabouraud Dextrose Broth</td>
<td>500 g</td>
<td>0382-17</td>
</tr>
<tr>
<td></td>
<td>100 g</td>
<td>0382-15</td>
</tr>
<tr>
<td></td>
<td>2 kg</td>
<td>0382-07</td>
</tr>
<tr>
<td>Sabouraud Maltose Agar</td>
<td>500 g</td>
<td>0110-17</td>
</tr>
<tr>
<td></td>
<td>2 kg</td>
<td>0110-07</td>
</tr>
<tr>
<td>Sabouraud Maltose Broth</td>
<td>500 g</td>
<td>0429-17</td>
</tr>
<tr>
<td>Fluid Sabouraud Medium</td>
<td>500 g</td>
<td>0642-17</td>
</tr>
</tbody>
</table>
**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. Bacto Schaedler Agar is used with or without blood in cultivating and enumerating anaerobic and aerobic microorganisms.

**Summary and Explanation**

Schaedler Agar and Schaedler Broth are prepared according to the formulation described by Schaedler, Dubos and Costello and modified by Mata, Carrillo and Villatoro. Modifications include reduced dextrose to avoid interference with hemolytic reactions and reduced yeast extract to avoid darkening of the medium 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. formulated a medium to recover both aerobic and anaerobic microorganisms. Mata et al. used a modification of the Schaedler formula to study human fecal microflora. Stalons, Thornsberry and Dowell 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% CO₂, 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. Since anaerobes vary in their sensitivity to oxygen and nutritional requirements, appropriate collection, culture medium and incubation are vital to recovery. Schaedler media are suitable for standard procedures used in cultivating anaerobic bacteria.

**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.
- Vitamin K₁ (1%) - to promote growth of some pigmented *Prevotella* and *Porphyromonas* spp. (formerly known as *Bacteroides*).
- Colistin and nalidixic acid (0.01 grams/liter, each) (Schaedler CNA agar) - for selectively isolating anaerobic gram-positive cocci.
- Kanamycin (0.01 grams/liter) and vancomycin (7.5 mg/liter) (Schaedler KV Agar) - for selectively isolating anaerobic gram-negative bacteria.

**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

**User Quality Control**

**Identity Specifications**

<table>
<thead>
<tr>
<th><strong>Schaedler Agar</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated Appearance:</td>
<td>Light tan, free-flowing, homogeneous.</td>
</tr>
<tr>
<td>Solution:</td>
<td>4.19% solution, soluble in distilled or deionized water on boiling. Light to medium amber, clear to slightly opalescent, may have a fine precipitate.</td>
</tr>
<tr>
<td>Prepared Medium:</td>
<td>Light to medium amber, clear to slightly opalescent, may have a fine precipitate.</td>
</tr>
<tr>
<td>Reaction of 4.19% Solution at 25°C</td>
<td>pH 7.6 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Schaedler Broth</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated Appearance:</td>
<td>Light tan, free-flowing, homogeneous.</td>
</tr>
<tr>
<td>Solution:</td>
<td>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.</td>
</tr>
<tr>
<td>Prepared Medium:</td>
<td>Light to medium amber, clear to very slightly opalescent, may have a very slight black precipitate.</td>
</tr>
<tr>
<td>Reaction of 2.84% Solution at 25°C:</td>
<td>pH 7.6 ± 0.2</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th><strong>ORGANISM</strong></th>
<th><strong>ATCC</strong></th>
<th><strong>INOCULUM CFU</strong></th>
<th><strong>GROWTH</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>25285T</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td><em>Bacteroides vulgatus</em></td>
<td>8482</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td><em>Clostridium novyi</em></td>
<td>27606</td>
<td>100-1,000</td>
<td>good</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>19615</td>
<td>100-1,000</td>
<td>good</td>
</tr>
</tbody>
</table>

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.
Schaedler Agar & Schaedler Broth

Section II

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

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

Packaging
Schaedler Agar 500 g 0403-17
Schaedler Broth 500 g 0534-17

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 K1 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.
**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. Although injured cells may not form colonies on selective media, they can cause infection if ingested. *Salmonella* spp. cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever. 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.

The formula of Selenite Broth is described by Leifson as Selenite F broth and Selenite Brilliant Green Mannitol (SBM) Enrichment Broth. Bacto Lactose is a fermentable carbohydrate. 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.

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 and USP 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*.

Selenite Broth conforms with APHA and is specified in Clinical Microbiology Procedures Handbook and Manual of Clinical Microbiology.

**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. This 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

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone</td>
</tr>
<tr>
<td>Bacto Lactose</td>
</tr>
<tr>
<td>Sodium Selenite</td>
</tr>
<tr>
<td>Sodium Phosphate</td>
</tr>
<tr>
<td>Final pH</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>CFU</th>
<th>GROWTH</th>
<th>MACCONKEY AGAR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>100-1,000</td>
<td>partial to marked inhibition</td>
<td>pink/wht ppt</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>14028*</td>
<td>100-1,000</td>
<td>good</td>
<td>colorless</td>
</tr>
</tbody>
</table>

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. **Very TOXIC. FATAL. IF INHALED OR SWALLOWED.**
   
   **VERY TOXIC BY INHALATION AND IF SWALLOWED.**
   
   **DANGER OF CUMULATIVE EFFECTS.**
   
   **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**


**Packaging**

<table>
<thead>
<tr>
<th>Selenite Broth</th>
<th>100 g</th>
<th>0275-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 g</td>
<td>0275-17</td>
<td></td>
</tr>
<tr>
<td>10 kg</td>
<td>0275-08</td>
<td></td>
</tr>
</tbody>
</table>

**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 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.3,4,5 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.  

**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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone</td>
</tr>
<tr>
<td>Bacto Lactose</td>
</tr>
<tr>
<td>Disodium Phosphate</td>
</tr>
<tr>
<td>Sodium Acid Selenite</td>
</tr>
<tr>
<td>L-Cystine</td>
</tr>
</tbody>
</table>

**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.

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCUM (CFU)</th>
<th>GROWTH</th>
<th>APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>100-1,000</td>
<td>partial</td>
<td>pink with bile precipitate</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>14028*</td>
<td>100-1,000</td>
<td>good</td>
<td>colorless</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>9290*</td>
<td>100-1,000</td>
<td>fair to</td>
<td>good colorless</td>
</tr>
</tbody>
</table>

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**

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.

**Test Procedure**

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

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 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 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, water samples, and food samples.

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.¹⁻³
2. Process each specimen, using procedures appropriate for that specimen or sample.¹⁻³

**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**

---

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC¹</th>
<th>GROWTH</th>
<th>COLONY COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter aerogenes</td>
<td>13048</td>
<td>good</td>
<td>blue</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>none to poor</td>
<td>green</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>14028</td>
<td>good</td>
<td>blue</td>
</tr>
</tbody>
</table>

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.

---

**Simmons Citrate Agar Section II**

7. FDA Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.


**Packaging**

<table>
<thead>
<tr>
<th>Simmons Citrate Agar</th>
<th>100 g</th>
<th>0091-15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 g</td>
<td>0091-17</td>
</tr>
</tbody>
</table>

---

## 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, including dairy products. 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).

### 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

<table>
<thead>
<tr>
<th>Material</th>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim Milk</td>
<td>100 g</td>
</tr>
</tbody>
</table>

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

Bacto 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.

### 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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC*</th>
<th>Growth</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus casei</td>
<td>9595</td>
<td>good</td>
<td>acid, reduction, curd</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>good</td>
<td>acid, reduction, curd</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>12919</td>
<td>good</td>
<td>stormy fermentation</td>
</tr>
</tbody>
</table>

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**

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**


**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 Agar¹

**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. Alban⁵  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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>GROWTH</th>
<th>ACID PRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus casei</em></td>
<td>9595</td>
<td>100-1,000</td>
<td>good</td>
<td>+</td>
</tr>
<tr>
<td><em>Lactobacillus fermentum</em></td>
<td>9338</td>
<td>100-1,000</td>
<td>good</td>
<td>+</td>
</tr>
</tbody>
</table>

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

---

¹ Snyder. ² Marshall. ³ Alban. ⁴ Snyder. ⁵ Alban.
**Formula**

**Snyder Test Agar**

**Formula Per Liter**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptose</td>
<td>20 g</td>
</tr>
<tr>
<td>Bacto Dextrose</td>
<td>20 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Bacto Agar</td>
<td>20 g</td>
</tr>
<tr>
<td>Brom Cresol Green</td>
<td>0.02 g</td>
</tr>
</tbody>
</table>

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. 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 Modification**

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:**

<table>
<thead>
<tr>
<th>Caries Activity</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked</td>
<td>Positive</td>
<td>Positive</td>
<td>–</td>
</tr>
<tr>
<td>Moderate</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Slight</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

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**

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

<table>
<thead>
<tr>
<th>TEST</th>
<th>SOLUTION OF SOYTONE OR SOYTONE NO. 2</th>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>SOYTONE RESULT</th>
<th>SOYTONE NO. 2 RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentable Carbohydrate 2%</td>
<td>Escherichia coli</td>
<td>25922*</td>
<td>positive</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>Indole Production 0.1%</td>
<td>Escherichia coli</td>
<td>25922*</td>
<td>positive</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>Acetylmethylcarbinol Production 1% w/0.5% NaCl and 0.5% dextrose</td>
<td>Enterobacter aerogenes</td>
<td>13048*</td>
<td>positive</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>Hydrogen Sulfide Production 1%</td>
<td>Salmonella typhimurium</td>
<td>14028*</td>
<td>positive</td>
<td>positive</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Ash (%)</th>
<th>Loss on Drying (%)</th>
<th>Clarity, 1% Solution (NTU)</th>
<th>pH, 1% Solution</th>
<th>Filterability (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.0</td>
<td>4.6</td>
<td>1.0</td>
<td>7.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Carbohydrate (%)

Total 24.0

Nitrogen Content (%)

Total Nitrogen 9.4 AN/TN 33.0
Amino Nitrogen 3.1

Amino Acids (%)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>2.46</th>
<th>3.45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>3.82</td>
<td>0.86</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>7.27</td>
<td>2.46</td>
</tr>
<tr>
<td>Cystine</td>
<td>1.45</td>
<td>2.92</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>12.76</td>
<td>2.87</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.51</td>
<td>2.17</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.24</td>
<td>0.47</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.37</td>
<td>1.93</td>
</tr>
<tr>
<td>Leucine</td>
<td>4.03</td>
<td>2.65</td>
</tr>
</tbody>
</table>

*These culture are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.
Inorganics (%)

- Calcium 0.055
- Chloride 0.165
- Cobalt <0.001
- Copper <0.001
- Iron 0.008
- Lead <0.001
- Magnesium 0.161
- Manganese <0.001

- Phosphate 0.820
- Potassium 2.220
- Sulfate 2.334
- Sulfur 1.660
- Tin <0.001
- Zinc 0.001

Vitamins (µg/g)

- Biotin 0.2
- Choline (as Choline Chloride) 2200.0
- Cyanocobalamin <0.1
- Folic Acid 3.0
- Inositol 2100.0
- Nicotinic Acid 19.1

- PABA 9.0
- Pantothenic Acid 13.0
- Pyridoxine 11.0
- Riboflavin <0.1
- Thiamine 1.2
- Thymidine 113.2

Biological Testing (CFU/g)

- Coliform negative
- Salmonella negative
- Spore Count 10

- Standard Plate Count 38
- Thermophile Count <3

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

<table>
<thead>
<tr>
<th>Soytone</th>
<th>500 g</th>
<th>0436-17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 kg</td>
<td>0436-08</td>
</tr>
<tr>
<td>Soytone No. 2</td>
<td>500 g</td>
<td>0508-17</td>
</tr>
<tr>
<td></td>
<td>10 kg</td>
<td>0508-08</td>
</tr>
</tbody>
</table>

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 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.

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.
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.

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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM</th>
<th>GROWTH</th>
<th>HALO/LIPOLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td>25933</td>
<td>100-1,000</td>
<td>good</td>
<td>no halo</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923*</td>
<td>100-1,000</td>
<td>good</td>
<td>halo</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>6538</td>
<td>100-1,000</td>
<td>good</td>
<td>halo</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>12228*</td>
<td>100-1,000</td>
<td>good</td>
<td>halo</td>
</tr>
</tbody>
</table>

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

Summary and Explanation
Staphylococci, along with other bacteria, are indicators of recreational water quality. Indicators of health risk include normal skin flora that are likely to be shed, such as Pseudomonas, Streptococcus, and Staphylococcus. These organisms account for a large percentage of swimming pool-associated illness.

The coagulase-positive species, Staphylococcus aureus, is well documented as a human opportunistic pathogen. Coagulase-negative Staphylococcus spp. are a major component of the normal microflora of humans. Staphylococci are widespread in nature, though they are mainly found living on the skin, skin glands, and mucous membranes of mammals and birds.

Chapman added 7.5% NaCl to Phenol Red Mannitol Agar to achieve a selective medium for staphylococci. While studying this medium formulation, Chapman 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.

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

<table>
<thead>
<tr>
<th>INGREDIENT</th>
<th>WEIGHT</th>
<th>CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto Tryptone</td>
<td>10 g</td>
<td></td>
</tr>
<tr>
<td>Bacto Yeast Extract</td>
<td>2.5 g</td>
<td></td>
</tr>
<tr>
<td>Bacto Lactose</td>
<td>2 g</td>
<td></td>
</tr>
<tr>
<td>Bacto Mannitol</td>
<td>10 g</td>
<td></td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
<td>5 g</td>
<td></td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>75 g</td>
<td></td>
</tr>
<tr>
<td>Final pH 7.0 ± 0.2 at 25°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: 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
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 or as specified by laboratory procedures.

Test Procedure
1. Follow the membrane filtration procedure described in Standard Methods, Section 9213, 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

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.

Summary and Explanation
Stone described a culture medium on which food-poisoning staphylococci gave a positive gelatinase test. Chapman, Lieb and Curcio later reported that pathogenic staphylococci strains typically ferment mannitol, form pigment and produce gelatinase. Chapman 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 led to the development of Staphylococcus Medium 110. This medium is included in standard methods procedures for selectively isolating pathogenic staphylococci from foods.

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).

**INOCULUM**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC⁷</th>
<th>CFU</th>
<th>GROWTH</th>
<th>PIGMENT*</th>
<th>Gelatin</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922*</td>
<td>100-300</td>
<td>marked to complete inhibition</td>
<td>–</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
| *These cultures are available as Bactrol” Disks and should be used as directed in Bactrol Disks Technical Information.

**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.

**Precautions**

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

**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.

**Test Procedure**

Consult appropriate references for procedures concerning selection and enumeration of staphylococci.

**Results**

Growth of pathogenic staphylococci produces colonies with yellow-orange pigment.

**Storage**

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

Packaging
Staphylococcus Medium
- 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, Veder 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.

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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC</th>
<th>Recovery</th>
<th>Starch Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>6633</td>
<td>good</td>
<td>positive</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922*</td>
<td>good</td>
<td>negative</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923*</td>
<td>good</td>
<td>negative</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>19615*</td>
<td>good</td>
<td>negative</td>
</tr>
</tbody>
</table>

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.

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

Packaging
Starch Agar 500 g 0072-17

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 Johnson1 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

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>25923*</td>
<td>good</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>6305</td>
<td>good</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>19615*</td>
<td>good</td>
</tr>
</tbody>
</table>

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

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

Bacto® Stock Culture Agar

Intended Use
Bacto Stock Culture Agar is used for maintaining stock cultures of bacteria, particularly streptococci.

Summary and Explanation
Ayers and Johnson1 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

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>25923*</td>
<td>good</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>6305</td>
<td>good</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>19615*</td>
<td>good</td>
</tr>
</tbody>
</table>

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
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

Packaging
Stock Culture Agar 500 g 0054-17

Bacto® Sulfite Agar

Intended Use
Bacto Sulfite Agar is used for detecting thermophilic, H₂S-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. Clark and Tanner 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. Desulfothrixium nigrificans, first classified as Clostridium nigrificans, causes spoilage in non-acid canned foods such as vegetables and infant formula. 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.

Sulfite Agar is a recommended Standard Methods medium for isolating D. nigrificans.

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**

**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.

**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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>INOCULUM CPU</th>
<th>GROWTH</th>
<th>SULFITE REDUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus stearothermophilus</td>
<td>10149</td>
<td>30-100</td>
<td>good</td>
<td>–</td>
</tr>
<tr>
<td>Clostridium thermosaccharolyticum</td>
<td>7956</td>
<td>30-100</td>
<td>good</td>
<td>+</td>
</tr>
<tr>
<td>Desulfotomaculum nigrificans</td>
<td>19858</td>
<td>30-100</td>
<td>good</td>
<td>+</td>
</tr>
</tbody>
</table>

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.
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. nigricans* 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.

**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**

**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). 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**

<table>
<thead>
<tr>
<th>Formula Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Cystine</td>
</tr>
<tr>
<td>DL-Methionine</td>
</tr>
<tr>
<td>L-Arginine HCl</td>
</tr>
</tbody>
</table>
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
L-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.

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

Packaging
Synthetic Broth AOAC 500 g 0352-17
10 kg 0352-08