I INTRODUCTION

Trypticase™ Soy Agar is a general purpose medium which supports the growth of fastidious as well as nonfastidious microorganisms.

II PERFORMANCE TEST PROCEDURE

1. Liquefy the medium in the tubed deeps by heating in boiling water. Cool to 45–50 °C, add 1 mL of sterile defibrinated sheep blood to two tubes (for inoculation with Streptococcus strains) and pour into sterile Petri dishes. Mix well to evenly distribute the blood throughout the medium and allow to solidify for a minimum of 30 min.

2. Inoculate representative samples with the cultures listed below.
   a. Using a 0.01 mL calibrated loop, inoculate the agar surfaces using 10⁻¹ dilutions of 18- to 24-h Trypticase Soy Broth cultures. Streak-inoculate the plates to assure the presence of well-isolated colonies.
   b. Incubate plates or tubed slants (with loosened caps) at 35 ± 2 °C in an aerobic atmosphere. Blood plates should be incubated in the presence of carbon dioxide.

3. Examine plates or tubes after 18–24 and 42–48 h for amount of growth and pigmentation. Examine the blood agar plates for hemolysis.

4. Expected Results

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC™</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Shigella flexneri</td>
<td>12022</td>
<td>Growth. Colonies medium to large, grayish-white, translucent, slightly convex and may be mucoid.</td>
</tr>
<tr>
<td>*Escherichia coli</td>
<td>25922</td>
<td>Growth</td>
</tr>
<tr>
<td>*Staphylococcus aureus</td>
<td>25923</td>
<td>Growth. Colonies medium to large, opaque, circular, entire with cream-yellow to gold pigment.</td>
</tr>
<tr>
<td>*Streptococcus pneumoniae</td>
<td>6305</td>
<td>Growth. Colonies surrounded by zones of alpha hemolysis (green).</td>
</tr>
<tr>
<td>*Streptococcus pyogenes</td>
<td>19615</td>
<td>Growth. Colonies surrounded by zones of beta hemolysis (clear to hazy).</td>
</tr>
</tbody>
</table>

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.3 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Trypticase Soy Agar is used for the isolation and cultivation of fastidious as well as nonfastidious microorganisms, including anaerobic as well as aerobic bacteria, although it is not the medium of choice for anaerobes.

V SUMMARY AND EXPLANATION

The nutritional composition of Trypticase Soy Agar has made it a popular medium for many years. It is the medium specified as Soybean-Casein Digest Agar Medium in The United States Pharmacopeia for the total aerobic microbial count portion of the microbial limit testing procedures.¹

The medium is used for a multitude of purposes including maintenance of stock cultures, plate counting, isolation of microorganisms from a variety of specimen types and as a base for media containing blood.²-⁴ It is included in the compendia of methods for the examination of water, wastewater and foods.⁵,⁶

VI PRINCIPLES OF THE PROCEDURE

The combination of casein and soy peptones in Trypticase Soy Agar renders the medium highly nutritious by supplying organic nitrogen, particularly amino acids and longer-chained peptides. The sodium chloride maintains the osmotic equilibrium.

VII REAGENTS

Trypticase Soy Agar

Approximate Formula* Per Liter Purified Water

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Papaic Digest of Soybean Meal</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.
Warnings and Precautions: For in vitro Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass. Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions”7–10 and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.3,11 Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Trypticase Soy Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Liquefy the medium in the tubed deeps by heating in boiling water. Cool to 45–50 °C, add blood, if desired, and pour into sterile Petri dishes. For general use, streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3–10% CO₂. Incubate plates at 35 ± 2 °C for 18–24 h.

Tubed slants of Trypticase Soy Agar are primarily used for the growth and maintenance of pure cultures. They should be inoculated with an inoculating loop and incubated under the same conditions as the plated medium.

User Quality Control: See “Quality Control Procedures.” Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a “dilution” technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. In addition, growth of each organism may be semiquantitatively scored on the basis of growth in each of the streaked areas. Hemolytic reactions should be noted for organisms inoculated on the medium containing blood. The tubed slant containing pure cultures can be used for additional studies or as stock cultures as desired.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.3,11,12

XII PERFORMANCE CHARACTERISTICS

Trypticase Soy Agar (TSA) with 5% Sheep Blood was used as a control in a study using broth-enhanced culture (Todd Hewitt) and Optical Immunoassay method for the diagnosis of b-hemolytic streptococcal infection. Five hundred two (502) specimens were tested. TSA with 5% Sheep Blood had a sensitivity and specificity of 92.5% and 99.4%, respectively.3 Nguyen et al. used Trypticase Soy Agar with 5% Sheep Blood as the ‘gold standard’ for the detection of group B Streptococcus from the lower genital tract of pregnant women.13 In another study, Rossmann et al. successfully reisolated Lautropia mirabilis on Trypticase Soy Agar with 5% Sheep Blood from the oral cavities of human immunodeficiency virus infected children.15 Of the 85 children evaluated in this study, 35 (41.4%) were positive for L. mirabilis. Isenberg et al. used Trypticase Soy Agar with 5% Sheep Blood as a control to evaluate the recovery of Enterococcus from a selective medium under study.16 Two hundred fifty (250) group D streptococcal strains isolated from clinical material and 8 strains obtained from the National Communicable Disease Center (Atlanta, Ga.) were used. Kantor et al. maintained stock cultures at room temperature using Trypticase Soy Agar slants covered with sterile mineral oil for a study on the identification of nonfermentative gram-negative bacteria in the clinical laboratory.17

XIII AVAILABILITY

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>221082</td>
<td>BBL™ Trypticase™ Soy Agar Deep (Pour Tubes), 20 mL, Pkg. of 10 size A tubes</td>
</tr>
<tr>
<td>221086</td>
<td>BBL™ Trypticase™ Soy Agar Slants, Pkg. of 10 size K tubes</td>
</tr>
<tr>
<td>221087</td>
<td>BBL™ Trypticase™ Soy Agar Slants, Ctn. of 100 size K tubes</td>
</tr>
</tbody>
</table>
XIV REFERENCES


Technical Information: In the United States contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.