QUALITY CONTROL PROCEDURES

I INTRODUCTION

Trypticase™ Soy Agar with 5% Horse Blood utilizes a highly nutritious base supplemented with horse blood.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak inoculate each plate with $10^3 - 10^4$ CFU of test organisms.
   b. Incubate the plates inoculated with Escherichia coli and Staphylococcus aureus at 35 ± 2 °C in an aerobic atmosphere. Incubate the streptococci and Haemophilus at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.

2. Examine plates after 18 – 24 h for amount of growth, colony size and hemolytic reactions.

3. Expected Results

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC™</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>19615</td>
<td>Growth, beta hemolysis</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>6305</td>
<td>Growth, alpha hemolysis</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923</td>
<td>Growth</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>Growth</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>10211</td>
<td>Moderate to heavy growth. Colonies non-hemolytic, grayish, small and translucent with a distinct “mousy odor.”</td>
</tr>
</tbody>
</table>

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates 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 $7.4 ± 0.2$.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Trypticase™ Soy Agar with 5% Horse Blood is used for the isolation and cultivation of nonfastidious and fastidious microorganisms.

V SUMMARY AND EXPLANATION

The nutritional composition of Trypticase™ Soy Agar (TSA) has made it a popular medium, both unsupplemented and as a base for media containing blood. Although this formulation supplemented with sheep blood is the most frequently used blood medium in clinical laboratories, some investigators prefer the use of horse blood. Trypticase™ Soy Agar with 5% Horse Blood, however, is not recommended for use with throat cultures.

VI PRINCIPLES OF THE PROCEDURE

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

Horse blood supplies both the X and V factors which are growth requirements for certain organisms, e.g., Haemophilus influenzae. Sheep and human blood are not suitable for this purpose because they contain enzymes which inactivate the nicotinamide adenine dinucleotide (NAD), which is the V factor. Defibrinated horse blood may give hemolytic reactions different from sheep blood. Some streptococci, e.g., group D, give hemolytic reactions on horse blood, but not on sheep blood and may be mistakenly reported as group A. If a hemolytic reaction is obtained, the organism should be tested with a Taxo™ A disc and it also should be grouped serologically or tested by the fluorescent method. Beta-hemolytic streptococci and Haemophilus hemolyticus may be differentiated by performing a Gram stain on a smear prepared from the colony.

VII REAGENTS

Trypticase™ Soy Agar with 5% Horse Blood

Approximate Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>14.5 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>Horse Blood, defibrinated</td>
<td>5%</td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For in vitro Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions” and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C until just prior to use may be inoculated.
up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking 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.9,10 Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

**IX PROCEDURE**

**Material Provided:** Trypticase Soy Agar with 5% Horse Blood

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

**Test Procedure:** Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

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 5% CO₂.

Incubate plates at 35 ± 2 °C for 18 – 72 h.

**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 (formerly NCCLS) 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 semi-quantitatively scored on the basis of growth in each of the streaked areas.

**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.9-14

**XII AVAILABILITY**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>221372</td>
<td>BBL™ Trypticase™ Soy Agar with 5% Horse Blood, Pkg. of 20 plates</td>
</tr>
</tbody>
</table>

**XIII REFERENCES**


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www.bd.com/ds

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