**Revisions**

<table>
<thead>
<tr>
<th>Rev from</th>
<th>Rev to</th>
<th>ECO #</th>
</tr>
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<tbody>
<tr>
<td>0703</td>
<td>2010/07</td>
<td>5393-10</td>
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</table>

**Notes:**

1. BD Cat. Number 297941, 297841
2. Blank (Sheet) Size: Length: 11” Width: 8.5”
   - Number of Pages: 2
   - Number of Sheets: 1
   - Page Size: Length 11” Width 8.5” Final Folded Size: 4.5” x 1.5”
3. Style (see illustrations below): #1

![Illustrations](image)

4. See Specification Control Number N/A for Material Information
5. Ink Colors: Printed two sides **Yes** **No**
   - No. of Colors: 1
   - PMS# Black
6. Graphics are approved by Becton, Dickinson and Company. Supplier has the responsibility for using the most current approved revision level
INTENDED USE

**Trypticase™ Soy Agar, Modified (TSA II)** supplemented with blood is used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

SUMMARY AND EXPLANATION

The nutritional composition of Trypticase Soy Agar has made it a popular medium, both unsupplemented and as a base for media containing blood. TSA II is an improved version of the original Trypticase Soy Agar formulation for use with animal blood supplements. With 5 or 10% sheep blood, it is extensively used for the recovery and cultivation of microorganisms and for the determination of hemolytic reactions that are important differentiating characteristics for bacteria, especially *Streptococcus* species.

**PRINCIPLES OF THE PROCEDURE**

The combination of casein and soy peptones renders the medium highly nutritious by supplying organic nitrogen, particularly amino acids and longer-chained peptides. The sodium chloride maintains osmotic equilibrium. Hemolytic reactions of streptococci are proper and growth of sodium chloride is inhibited. Defibrinated sheep blood is the most widely used blood for enriching agar base media. Hemolytic reactions of streptococci are proper and growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

**REAGENTS**

**Trypticase™ Soy Agar, Modified (TSA II)**

**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>Papain 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>14.0 g</td>
</tr>
<tr>
<td>Growth Factors</td>
<td>0.5 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.

**Storage Instructions:** On receipt, store tubes in the dark at 2 to 25°C. Avoid freezing and overheating. Do not open until ready to use. 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. Minimize exposure to light.

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

**SPECIMEN COLLECTION AND HANDLING**

Specimens suitable for culture may be obtained using various techniques. For detailed information, consult appropriate texts. Specimens should be obtained before the specimen arrives at the laboratory. Pathogenic microorganisms, including hepatitis virus 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. Prior to discarding, sterile specimen containers and other contaminated materials must be autoclaved.

**PROCEDURE**

**Material Provided:** Trypticase Soy Agar, Modified (TSA II)

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

**Test Procedure:** Obtain aseptic techniques. To prepare plated medium, place agar deeps with loosened caps in a boiling water bath until the medium becomes liquid (clear). Cool to 45 to 50°C, add blood, if desired, and pour into sterile Petri dishes. Allow the medium to solidify and dry before use. The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at 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 to 10% CO₂. Incubate plates at 35 ± 2°C for 18 to 24 h.

**User Quality Control:**

1. Examine the tubes for signs of deterioration as described under "Product Deterioration".
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that give known, desired reactions. The following test strains are recommended:

<table>
<thead>
<tr>
<th>Test Strain</th>
<th>Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium without the addition of blood.</td>
<td>Growth</td>
</tr>
<tr>
<td><em>Shigella flexneri</em> ATCC™ 12022</td>
<td>Growth</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>Growth</td>
</tr>
<tr>
<td>Medium with the addition of 5% defibrinated sheep blood.</td>
<td>Growth</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> ATCC 6305</td>
<td>Growth, Colonies surrounded by zones of alpha hemolysis (green).</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> ATCC 19615</td>
<td>Growth, Colonies surrounded by zones of beta hemolysis.</td>
</tr>
</tbody>
</table>

**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 NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

**RESULTS**

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Hemolytic reactions should be noted for organisms inoculated on the medium containing blood.

**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 tests for detailed information and recommended procedures.

**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 β-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. 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. 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. 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. 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.

**AVAILABILITY**

Cat. No. Description

<table>
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<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>297941</td>
<td>BBL™ Trypticase™ Soy Agar, Modified (TSA II) Deeps, 20 mL, Ctn. of 100 size A tubes</td>
</tr>
<tr>
<td>297841</td>
<td>BBL™ Trypticase™ Soy Agar, Modified (TSA II) Deeps, 9 mL, Ctn. of 100 size D tubes</td>
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</tbody>
</table>
REFERENCES


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