BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)
BBL™ Trypticase™ Soy Agar with 10% Sheep Blood

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QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Trypticase Soy Agar supplemented with sheep blood is used for the growth of fastidious organisms and for the visualization of hemolytic reactions.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Using a volumetric pipettor or equivalent method, deliver 0.1 mL of a dilution yielding 30–300 CFU (for all products with exception of catalog no. 221162) to each plate and spread-inoculate using a sterile glass spreader.

      NOTE: Streak inoculate catalog no. 221162, TSA with 10% Sheep Blood, with 0.01 mL of a dilution yielding 10^3–10^4 CFUs for all organisms.
   b. Incubate the *Staphylococcus* and *Escherichia* strains at 35 ± 2 °C in an aerobic atmosphere and the *Streptococcus* strains at 35 ± 2 °C in an aerobic atmosphere supplemented with 3–5% carbon dioxide.
2. Examine plates after 18–24 h for growth, colony size and hemolytic reactions.
3. Expected Results

CLSI Organisms	ATCC®	Recovery
**Streptococcus pyogenes*	19615	Growth, beta hemolysis
**Streptococcus pneumoniae*	6305	Growth, alpha hemolysis
**Staphylococcus aureus*	25923	Growth
**Escherichia coli*	25922	Growth

*Recommended organism strain for User Quality Control.

III TEST FOR CAMP REACTION

(Not performed on plates containing 10% blood.)

1. Inoculate representative samples with 18-to 24-h **Trypticase** Soy Agar with 5% Sheep Blood (TSA II) cultures of the organisms listed below.
   a. Identify the plates by denoting the *Staphylococcus aureus* ATCC 25923 streak as a long line across the width of the plate. Denote streptococcal cultures by the respective numbers perpendicular to the staphylococcal lines.
   b. Using an inoculating loop, make a single narrow streak inoculation across the width of each plate with the *S. aureus* culture and allow the streak to dry. Make a narrow streak with each streptococcal culture perpendicular to, but not touching (within 2–3 mm of) the *S. aureus* streak. Perpendicular streaks should be at least 5 mm apart.
   c. Incubate plates at 35 ± 2 °C in an aerobic atmosphere.
2. Examine plates after 18–24 h.
3. Expected Results

Organisms	ATCC	Reaction
**Streptococcus agalactiae* (Group B)	12386	A typical arrowhead or crescent-shaped clearing should occur at the junction of the *Streptococcus* and *S. aureus* streaks within 24 h.
Streptococcus pyogenes	19615	No arrowhead formation. (A bullet-shaped zone of slight hemolysis may appear at the junction of the two streaks.)

IV 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 7.3 ± 0.2 for catalog numbers 221239 and 221261 and 7.4 ± 0.2 for catalog number 221162.
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

V INTENDED USE

Trypticase Soy Agar with 5% or 10% Sheep Blood is used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species. Plates labeled “deep fill” contain an additional volume of medium to reduce the effects of drying during prolonged incubation.

VI 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. **Trypticase** Soy Agar with 5% or 10% Sheep Blood is extensively used for the recovery and cultivation of fastidious microbial species and for the determination of hemolytic reactions which are important differentiating characteristics for bacteria, especially *Streptococcus* species.

VII PRINCIPLES OF THE PROCEDURE

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

Defibrinated sheep blood is the most widely used blood for enriching agar base media.[1] 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.

Trypticase Soy Agar with 5% Sheep Blood (TSA II) provides excellent growth and beta hemolysis by *Streptococcus pyogenes* (Lancefield group A) and also provides excellent growth and appropriate hemolytic reactions with other fastidious organisms. It is suitable for performing the CAMP test for the presumptive identification of group B streptococci (*S. agalactiae*), and for use with low concentration (0.04 unit) bacitracin discs (**Taxo™** A) for presumptive identification of group A streptococci (*S. pyogenes*).

The CAMP test, which only is performed on TSA II, is based on the formation of a zone of synergistic hemolysis at the junction of perpendicular streak inocula of *Staphylococcus aureus* and group B streptococci. The reaction is caused by the sphingomyelinase (beta-toxin) of *S. aureus* reacting with sphingomyelin in the sheep erythrocyte membrane to produce ceramide. A non-enzymatic protein (CAMP protein), produced by *S. agalactiae*, binds to the ceramide and leads to disorganization of the lipid bilayer of the sheep erythrocyte membrane resulting in complete lysis.[2]

Trypticase Soy Agar with 10% Sheep Blood is provided for those laboratories preferring the increased blood content. This medium is not recommended for performance of the CAMP test. Additionally, the increased blood content can make hemolytic reactions less distinct and more difficult to read.

I Plate™ divided Petri dishes containing **Trypticase** Soy Agar with 5% Sheep Blood in each half enable two specimens to be streaked on one plate.

VIII REAGENTS

Trypticase Soy Agar with 5% or 10% Sheep Blood

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	14.5 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Agar	14.0 g
Growth Factors	1.5 g
Defibrinated Sheep Blood	5% or 10%

*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"[3-6] 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.

IX SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[7,8] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

X PROCEDURE

Material Provided: TSA II (**Trypticase** Soy Agar with 5% Sheep Blood) or **Trypticase** Soy Agar with 10% Sheep Blood or **Trypticase** Soy Agar with 5% Sheep Blood (TSA II) - **I Plate**

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 3–10% CO_2.[9]

Incubate plates at 35 ± 2 °C for 18–24 h.

CAMP Test[10]

Non-hemolytic, bile-esculin negative streptococci or bacitracin-resistant beta-hemolytic streptococci may be tested by the CAMP test for presumptive identification as *S. agalactiae* (Lancefield group B). The inoculum may be taken from an overnight broth culture or from colonies picked from a blood agar plate. Make a single streak of *Staphylococcus aureus* ATCC 25923 or ATCC 33862 across the center of a TSA II plate. If a loop is used, do not use it parallel to the agar surface, since the streak will be too wide and the results will not be satisfactory. The streptococcal isolates to be tested are inoculated by making a simple streak perpendicular to the *S. aureus* line coming as close as possible (2–3 mm), but not touching it. Several streptococcal isolates may be tested on the same plate. Perpendicular streptococcal streaks should be 5–8 mm apart. Include a known *S. agalactiae* for a positive control and *S. pyogenes* as a negative control. The procedure should be practiced with known cultures before using it to identify unknown isolates.

NOTE: Studies on the CAMP Test have shown that the reaction is most reliable early in the shelf life of some lots of the prepared plated medium. It is recommended that *S. agalactiae* ATCC 12386 be included along with patient isolates to verify satisfactory performance.

Incubate plates in an aerobic atmosphere at 35 ± 2 °C for 18–24 h. Do not incubate anaerobically or in a CO_2 incubator. False-positive results may occur with group A streptococci when incubation is in an anaerobic or CO_2-enriched atmosphere.[10,11]

User Quality Control: See "Quality Control Procedures."

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

XI 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. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

1. Hemolytic streptococci may appear as translucent or opaque, grayish, small (1 mm), or large matt and mucoid (2–4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.)

   In reporting, approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.
2. CAMP Test - A positive CAMP reaction is indicated by arrowhead or triangular shaped area of increased hemolysis which forms around the end of the streptococcal streak line closest to the *S. aureus* growth. The streptococcal growth must be within the wide zone of partial hemolysis that surrounds the *S. aureus* growth. A negative reaction may appear as a bullet-shaped zone of slightly increased hemolysis or as no increased hemolysis.

   Bacitracin-negative, CAMP-positive, beta-hemolytic streptococci may be reported as presumptive group B streptococci. CAMP-positive group A species may be differentiated from group B streptococci by hemolysis, bacitracin susceptibility, and hippurate hydrolysis. Group B streptococci generally have smaller hemolytic zones than group A streptococci.[11]
3. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.
4. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
5. *Listeria*. Small zones of beta hemolysis are produced. They may be distinguished by their rod shape in stains, and by motility at room temperature.
6. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

XII 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.[7,8,12-15]

XIII PERFORMANCE CHARACTERISTICS

Trypticase Soy Agar with 5% Sheep Blood

Trypticase Soy Agar 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 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.[16] 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.[17] 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.[18] Of the 85 children evaluated in the 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.[19] 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.

XIV AVAILABILITY

Cat. No.	Description
221239	**BD BBL™ Trypticase™** Soy Agar with 5% Sheep Blood (TSA II), Pkg. of 20 plates
221261	**BD BBL™ Trypticase™** Soy Agar with 5% Sheep Blood (TSA II), Ctn. of 100 plates
221162	**BD BBL™ Trypticase™** Soy Agar with 10% Sheep Blood, Pkg. of 20 plates
215372	**BD BBL™ Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) Alt Sleeve, Ctn. of 100 plates

XV REFERENCES

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