BBL™ Group A Selective Strep Agar with 5% Sheep Blood (ssA™) and BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)–Bi-Plate

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R_x Only

QUALITY CONTROL PROCEDURES

I INTRODUCTION

BD BBL™ Group A Selective Strep Agar with 5% Sheep Blood (**ssA**) is a selective medium for use in the isolation and presumptive identification of group A streptococci from throat cultures and other specimens. **BD BBL™ Trypticase** Soy Agar with 5% Sheep Blood (TSA II) is used for the growth of fastidious organisms and for the visualization of hemolytic reactions. The TSA II medium sector is marked "I" and the **ssA** medium sector is marked "II" in the bi-plate dish.

II PERFORMANCE TEST PROCEDURE

A. BD BBL Group A Selective Strep Agar with 5% Sheep Blood

1. Inoculate representative samples with the cultures diluted to contain 10^3–10^4 CFU/0.01 mL.
   a. To each plate, add 0.01 mL of the dilution and streak for isolation. Make a stab in the primary streak area before streaking the rest of the plate.
   b. Place a **BD BBL Taxo™** A disc at the intersection of the first and second area of streaking on all plates inoculated with *S. pyogenes* strains.
   c. Incubate plates at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.
   d. Include **BD BBL Trypticase** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for beta hemolysis in the stabbed area and for amount of growth, inhibition, colony size and hemolytic reactions. Read and record the size of the zone around the **BD BBL** Taxo A disc with *S. pyogenes*.
3. Expected Results

Organisms	ATCC®	Recovery
**Streptococcus pyogenes*	19615	For both strains of *S. pyogenes,* fair to heavy growth (depending on the strain and dilution) of pinpoint to very small colonies surrounded by zones of beta hemolysis. A zone of growth inhibition is clearly evident around the **BD BBL Taxo** A disc.
Streptococcus pyogenes	51574	
Streptococcus mitis	6249	Partial inhibition
**Staphylococcus aureus*	25923	Complete inhibition
Neisseria subflava	14799	Complete inhibition
Pseudomonas aeruginosa	27853	Complete inhibition

*Recommended organism strain for User Quality Control.

B. BD BBL Trypticase Soy Agar with 5% Sheep Blood

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Using a volumetric pipettor or equivalent method, deliver 0.01 mL of a dilution yielding 30–300 CFU to each plate and spread-inoculate using a sterile glass spreader.
   b. Incubate the *Staphylococcus* strain at 35 ± 2 °C in an aerobic atmosphere and the *Streptococcus* strains at 35 ± 2 °C in an aerobic atmosphere supplemented with 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 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 for both media.
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

BD BBL Group A Selective Strep Agar with 5% Sheep Blood (**ssA**) is recommended as a primary selective plating medium for the primary isolation of group A streptococci (*S. pyogenes*) from throat cultures and other specimens in which the presence of *S. pyogenes* is suspected. Group B streptococci will also grow on this medium; most other streptococci, neisseriae, staphylococci and gram-negative bacteria are inhibited. The medium is designed for use in conjunction with **BD BBL Taxo** A (bacitracin, 0.04 unit) discs for presumptive identification of *S. pyogenes*.

BD BBL Trypticase Soy Agar with 5% Sheep Blood (TSA II) is used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

V SUMMARY AND EXPLANATION

Infection with Lancefield group A streptococci (*S. pyogenes*) may produce serious sequelae such as rheumatic fever and acute glomerulonephritis. Therefore, early detection and identification are important.

The nutritional composition of **BD BBL Trypticase** Soy Agar has made it a popular medium, both unsupplemented and as a base for media containing blood. **BD BBL Trypticase** Soy Agar with 5% Sheep Blood (TSA II) 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.

Because of the overgrowth of normal flora present in throat culture specimens plated on routine blood agar plates, selective ingredients have been added to sheep blood agar to enhance the detection of group A streptococci.

Evaluation of various antimicrobial agents in our laboratories resulted in a combination with improved selectivity over other selective media tested. This medium (**ssA**) allows presumptive identification of group A streptococci, based on bacitracin susceptibility and beta hemolysis, within 24 h after inoculation with the specimen when the medium is incubated in a CO_2-enriched atmosphere.[1]

The divided bi-plate, containing the nonselective blood agar (TSA II) in the sector marked "I" and the selective blood agar (**ssA**) in the sector marked "II," permits the recovery of group A streptococci and evaluation of the total specimen microbiota with one dish.

VI PRINCIPLES OF THE PROCEDURE

The combination of casein and soy peptones in the **BD BBL Trypticase** Soy Agar base renders the medium highly nutritious by supplying organic nitrogen. The sodium chloride maintains osmotic equilibrium.

Defibrinated sheep blood provides proper hemolytic reactions of streptococci. In addition, growth of *Haemophilus haemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

BD BBL 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 use with low concentration (0.04 unit) bacitracin discs (**BD BBL Taxo** A) for presumptive identification of group A streptococci (*S. pyogenes*).

BD BBL Group A Selective Strep Agar with 5% Sheep Blood (**ssA**) incorporates a unique combination of selective ingredients in **BD BBL Trypticase** Soy Sheep Blood Agar (TSA II) to suppress normal throat flora for improved recovery of *S. pyogenes*. Defibrinated sheep blood supplies enrichment for the growth of such fastidious organisms and allows detection of the typical beta hemolysis of *S. pyogenes*. Beta-hemolytic streptococci which show a zone of inhibition around a bacitracin (0.04 unit) disc may be presumptively identified as group A streptococci.

VII REAGENTS

BD BBL Group A Selective Strep Agar with 5% Sheep Blood (ssA)		**BD BBL Trypticase Soy Agar with 5% Sheep Blood (TSA II)**	
Approximate Formula* Per Liter Purified Water		Approximate Formula* Per Liter Purified Water	
Pancreatic Digest of Casein	14.5 g	Pancreatic Digest of Casein	14.5 g
Papaic Digest of Soybean Meal	5.0 g	Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g	Sodium Chloride	5.0 g
Agar	14.0 g	Agar	14.0 g
Growth Factors	1.5 g	Growth Factors	1.5 g
Selective Agents	40.2 mg	Defibrinated Sheep Blood	5%
Sheep Blood, defibrinated	5%		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert 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"[2-5] 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 or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Throat specimens suitable for culture may be obtained by swabbing the pharynx and tonsillar area of the throat with a polyester- or polyurethane-tipped swab, taking care to avoid touching the tongue or uvula. (Note: if swabs are also used with direct antigen detection tests, the use of polyester, rayon or polyurethane swabs on plastic shafts is required; e.g., **BD BBL CultureSwab™** and **BD BBL CultureSwab™** EZ Collection and Transport Systems.) Sources other than the throat should be cultured according to recommended procedures. For detailed information, appropriate texts should be consulted.[6,7]

IX PROCEDURE

Material Provided: BD BBL Group A Selective Strep Agar with 5% Sheep Blood (**ssA**) and **BD BBL Trypticase** Soy Agar with 5% Sheep Blood (TSA II)–Bi-Plate.

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

Test Procedure: 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. Without resterilizing the loop, stab the agar two or three times in the areas of heaviest inoculation.

When using this plate with the same specimen, inoculate the TSA II side, marked "I", first. Then inoculate the **ssA** side, marked "II", and place a **BD BBL Taxo** A disc on the swabbed portion of that side; i.e., where the swabbed area is intersected by the area of initial loop streaking.

Incubate inoculated plates at 35 ± 2 °C in an atmosphere enriched with carbon dioxide. If plates are incubated without carbon dioxide, the beta-hemolytic zones and colony size will be smaller and fewer colonies may be apparent.

Examine plates after 18–24 h.

User Quality Control:

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 18–24 h of incubation in an atmosphere enriched with carbon dioxide, group A streptococci (*S. pyogenes*) on **ssA** will appear as translucent or opaque, white to gray, small (1–2 mm) colonies surrounded by a zone of beta hemolysis. A decrease in size as compared to the nonselective control, **BD BBL Trypticase** Soy Agar with 5% Sheep Blood, is typical. Pinpoint or very small colonies of alpha- , nonhemolytic or other beta-hemolytic streptococci may grow in small numbers, but they should not interfere with the recovery of group A streptococci or interpretation of the results. *Neisseria* species, viridans streptococci, staphylococci, gram-negative rods and most beta-hemolytic streptococci other than groups A and B are inhibited on the **ssA** medium. Bacitracin susceptibility may be used to differentiate group A streptococci from group B. Fair to heavy growth of beta-hemolytic colonies demonstrating a zone of inhibition around the **BD BBL Taxo** A disc may be presumptively reported as *S. pyogenes*. A PYR (pyroglutamic acid) test may also be performed. It is more specific and as sensitive as the bacitracin test for this purpose.[7] Gram stains should be made and examined.

A serological grouping test procedure may be performed if sufficient well-isolated beta-hemolytic colonies are present.

XI LIMITATIONS OF THE PROCEDURE

Since there is no such entity as a perfect medium, some strains of group A streptococci (*S. pyogenes*) may be encountered that will grow poorly on the **ssA** medium; the nature of the specimens and the physiologic state of the organisms can influence recovery of the desired species, as well as modify the effects of the inhibitory characteristics of the medium. It is therefore useful to compare the growth on both sides of the bi-plate to obtain additional information and to assure optimal recovery of any potential pathogens.

This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for detailed information and recommended procedures.[6-8]

XII PERFORMANCE CHARACTERISTICS

In a clinical evaluation consisting of 460 throat cultures, there was a total of 117 positive for group A streptococci on the **BD BBL** Group A Selective Strep Agar with 5% Sheep Blood (**ssA**) compared to 100 on SXT Sheep Blood Agar and 84 on **BD BBL Trypticase** Soy Agar with 5% Sheep Blood (TSA II). Of these positive cultures, 103 were identified based on beta hemolysis and bacitracin (0.04 unit) susceptibility within 24 h with **ssA** compared with 80 on SXT and only 32 on the nonselective TSA blood agar control.[9]

XIII AVAILABILITY

Cat. No.	Description
221783	**BD BBL™** Group A Selective Strep Agar with 5% Sheep Blood (**ssA™**) // **BD BBL™ Trypticase™** Soy Agar with 5% Sheep Blood (TSA II)

XIV REFERENCES

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Technical Information: In the United States contact BD Technical Service and Support at 1.800.638.8663 or www.bd.com.

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