



# BBL™ Group A Selective Strep Agar with 5% Sheep Blood (ssA™)

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## QUALITY CONTROL PROCEDURES

### I INTRODUCTION

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.

### II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures diluted to contain  $10^3$ – $10^4$  CFU/0.01 mL.
  - To each plate or sector, add 0.01 mL of the dilution and streak for isolation. Stab the agar in the primary streak area before streaking the rest of the plate.
  - Place a **Taxo™** A disc at the intersection of the first and second areas of streaking on all plates inoculated with *S. pyogenes* strains.
  - Incubate plates at  $35 \pm 2^\circ\text{C}$  in an aerobic atmosphere supplemented with 3–7% carbon dioxide.
  - Include **Trypticase™** Soy Agar with 5% Sheep Blood Agar (TSA II) plates as nonselective controls for all organisms.
- 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 **Taxo** A disc with *S. pyogenes*.
- Expected Results

Organisms	ATCC™	Recovery
* <i>Streptococcus pyogenes</i>	19615	For both strains, 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 <b>Taxo</b> A disc.
<i>Streptococcus pyogenes</i>	51574	
<i>Streptococcus mitis</i>	6249	Partial inhibition
* <i>Staphylococcus aureus</i>	25923	Complete inhibition
<i>Neisseria subflava</i>	14799	Complete inhibition
<i>Pseudomonas aeruginosa</i>	27853	Complete inhibition

\*Recommended organism strain for User Quality Control.

### III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of  $7.4 \pm 0.2$ .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at  $35 \pm 2^\circ\text{C}$  in an aerobic atmosphere supplemented with 3–7%  $\text{CO}_2$  for 72 h and examine for microbial contamination.

## PRODUCT INFORMATION

### IV INTENDED USE

Group A Selective Strep Agar with 5% Sheep Blood (ssA) is recommended as a primary selective plating medium for the 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 **Taxo** A (bacitracin, 0.04 unit) discs for presumptive identification of *S. pyogenes*.

### 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.

Because of the overgrowth of normal flora present in throat culture specimens inoculated onto 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  $\text{CO}_2$ -enriched atmosphere.<sup>1</sup>

The JEMBEC™ system offers a convenient  $\text{CO}_2$  atmosphere for an individual plate. It consists of the selective culture medium (ssA) in a rectangular plate with a special well to hold a  $\text{CO}_2$ -generating tablet. A resealable plastic bag is provided to maintain the  $\text{CO}_2$ -enriched environment during incubation. The system may be inoculated and incubated at the site of specimen collection or used for specimen transport and growth of *S. pyogenes*. It has the advantage over other transport systems of obviating the necessity of transferring the specimen from the transport system to a culture plate.

### VI PRINCIPLES OF THE PROCEDURE

The base medium, modified **Trypticase** Soy Agar (TSA II), provides a combination of casein and soy peptones that supply organic nitrogen. The sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood provides proper hemolytic reactions of streptococci. In addition, growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited. The incorporation of a unique combination of selective ingredients provides improved suppression of normal throat flora for improved recovery 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.

In the JEMBEC system, a CO<sub>2</sub>-generating tablet is placed in a well within the plate and is activated by the moisture produced by the culture medium within the sealed plastic bag. The CO<sub>2</sub> level generated is sufficient for the growth of *S. pyogenes* on the selective medium provided with the system.

## VII REAGENTS

### Group A Selective Strep Agar with 5% Sheep Blood (ssA)

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
Selective Agents.....	40.2 mg
Sheep Blood, defibrinated .....	5%

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

### JEMBEC System

In addition to the plated medium, the JEMBEC System consists of resealable polyethylene bags and CO<sub>2</sub>-generating tablets (sodium bicarbonate and citric acid).

### 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"<sup>2-5</sup> 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

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., **CultureSwab™** Collection and Transport Systems. Sources other than the throat should be cultured according to recommended procedures. For detailed information, appropriate texts should be consulted.<sup>6,7</sup> Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Group A Selective Strep Agar with 5% Sheep Blood (ssA)

**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.

As soon as possible, inoculate the specimen onto a Group A Selective Strep Agar with 5% Sheep Blood (ssA) plate by firmly rolling the swab over a third of the agar surface. Streak the remainder of the plate with a sterilized or sterile disposable inoculating loop to obtain isolated colonies. After streaking, stab the agar two or three times in the area of heaviest inoculation.

Place a **Taxo A** disc directly on the swabbed portion of the plate; i.e., where the swabbed area is intersected by the area of initial loop streaking. It is recommended that a routine blood agar plate, such as a **Trypticase Soy Agar with 5% Sheep Blood plate (TSA II)** also be inoculated to assure the recovery of microorganisms that may be inhibited on the selective medium.

Incubate inoculated plates at 35 ± 2°C in an atmosphere enriched with 3–7% 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.

For the JEMBEC system:

1. Hold the plate firmly in either hand with the tablet-well turned toward you.
2. Lift the lid so that the plate may be held open with one hand.
3. Beginning at one end of the rectangular plate, inoculate one-third the length of the medium surface. Spread the inoculum over the remainder of the medium surface with a sterilized or sterile disposable loop to obtain isolated colonies.
4. Place a **Taxo A** disc as described above.

To generate CO<sub>2</sub>:

1. Open foil package and remove the tablet with forceps.
2. Place the tablet into the well.
3. Close the lid.

- Place the plate in the bag provided and seal securely. It is not necessary to moisten or add water to the CO<sub>2</sub>-generating tablet; the moisture is supplied by the medium during incubation.

Incubate for 18–24 h at 35 ± 2°C.

**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 18–24 h of incubation in an atmosphere enriched with carbon dioxide, group A streptococci (*S. pyogenes*) will appear as translucent or opaque, white to gray, small (1 to 2 mm) colonies surrounded by a zone of beta hemolysis. A decrease in size as compared to a nonselective control 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. 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 **Taxo A** disc on this medium 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.<sup>8</sup> 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 this 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 this medium. It is therefore useful to examine nonselective controls and compare them to the selective medium 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.<sup>6,7,9-12</sup>

## 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 Group A Selective Strep Agar with 5% Sheep Blood (**ssA**) compared to 100 on SXT Sheep Blood Agar and 84 on **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.<sup>13</sup>

## XIII AVAILABILITY

Cat. No.	Description
221779	<b>BBL™</b> Group A Selective Strep Agar with 5% Sheep Blood ( <b>ssA™</b> ), Pkg. of 20 plates
221780	<b>BBL™</b> Group A Selective Strep Agar with 5% Sheep Blood ( <b>ssA™</b> ), Ctn. of 100 plates
221936	<b>BBL™</b> Group A Selective Strep Agar with 5% Sheep Blood ( <b>ssA™</b> ), Pkg. of 20 <b>JEMBEC™</b> plates

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